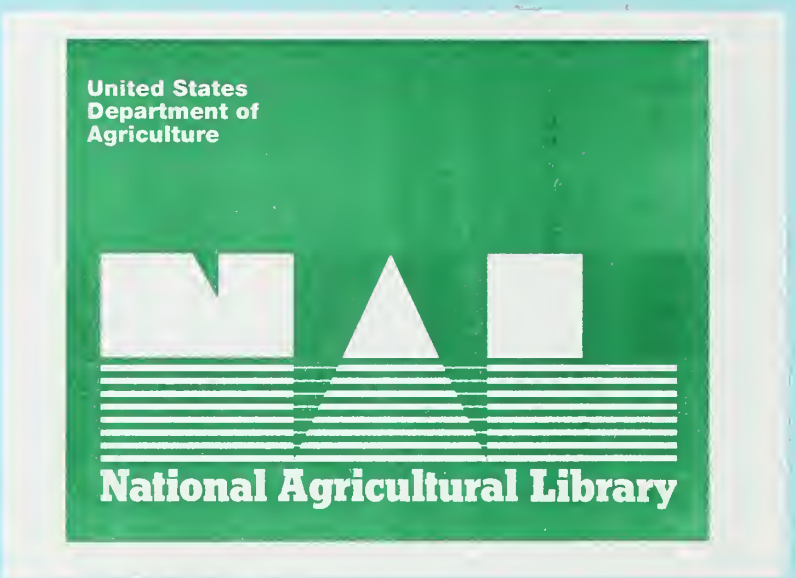
United States Department of Agriculture
Agricultural Research Service

BIOLOGICALLY ACTIVE PEPTIDE RESEARCH WORKSHOP

October 31-November 1, 1984
Beltsville, Maryland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Acknowledgments

Successful meetings are dependent upon the effort and willingness of those individuals who give freely of their time and effort. Such is indeed the norm for this Biologically Active Peptide Research Workshop. Dr. A. Borkovec was instrumental in organizing the workshop and coordinating on-site laboratory visits for the participants. Dr. R. J. Gerritts, Dr. D. D. King, Dr. R. D. Jackson, and Dr. M. H. Rogoff suggested participants and topics of discussion. Mrs. D. Drzymuchowski provided the extensive secretarial needs necessary for the resulting report and her efforts are very much appreciated.

The group discussion leaders, Dr. T. J. Kelly, Dr. H. Oberlander, Dr. A. Chen and Dr. E. Marks contributed significantly with their overall leadership and in development of the report.

This workshop report represents an effort to develop a current profile on the program and the responsible scientists. Your continued input and research accomplishments are solicited to provide a continuous update to this document and also your suggestions on how the program can be improved.

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TABLE OF CONTENTS

Executive Summary

Research on peptides in ARS covers a wide range of programs reflecting the extensive distribution of these molecules in nature and their importance in all aspects of agriculture. At the ARS Peptide Workshop III, representatives were present from the areas of insect reproduction, veterinary toxicology and entomology, livestock insects, insect physiology and metabolism, insect behavior, animal reproduction, animal physiology, animal parasitology, animal virology, plant biology, and plant protection. These programs included substantial cooperation within ARS and with numerous industrial, academic and medical institutions, both nationally and internationally.

ARS effort in peptide research is approximately 30 SY's. Although there is a significant overlap in various areas, best estimates indicate that the primary emphasis is on peptide neurohormones for:

-- regulation of reproduction of farm animals and insects (10 SY'S)
-- regulation of insect physiology and metabolism (4 SY'S)
-- regulation of insect behavior (3 SY'S)

Other areas of emphasis are:

-- chemical characterization and isolation of peptides (3 SY'S)
-- insect cell and parasite growth factors (2.5 SY'S)
-- immune responses to virus and parasite peptides (2 SY'S)
-- insect diapause (2 SY'S)
-- chemical modifiers of insect peptide neurohormone related processes (1 SY)
-- comparative studies of vertebrate and neurohormone related processes (1 SY)
-- insect neuropharmacology (0.5 SY)
-- plant mycotoxin peptides (0.5 SY)

Most of these programs have been developing over the last 5-10 years with significant emphasis on peptides only in the last few years. Many programs are oriented toward isolating specific peptides and determining their physiological controls and interactions by utilizing up-to-date analytical and biochemical separatory techniques, such as high-pressure and affinity chromatography, and developing biological, immunological and molecular genetic assays.

Current research accomplishments include significant progress in:

-- egg development neurosecretory hormone (EDNH) in mosquitos (Insect Reproduction Lab., Beltsville, Md)
-- in glycoprotein inhibitory hormone (inhibin) from the Ovarian follicular fluid of cows and pigs (Reproduction Laboratory, Beltsville, Md)
-- an immune-response activating region from the protein coat of foot and mouth disease virus (Plum Island Animal Disease Center, Greenport, NY).

Peptide molecular biology and immunological capabilities have been pursued cooperatively in a few groups and are now being established as permanent in-house programs by some. Peptide receptor recognition and interaction studies are preliminary in many groups and neurophysiological studies are minimal, at best.

Overall, with the expertise present at this workshop, it can generally be stated that the strong points of the ARS peptide research program are in the area of peptide neurohormones, especially peptide neurohormones that regulate the reproduction of farm animals and insects. This gives ARS a strong position from which to carry out cross-species correlations between vertebrate, invertebrate, and possibly even plant peptides. These studies will eventually be necessary for estimating the selectivity of peptide-based animal growth enhancement and pest control procedures and for determining the safety of releasing these agents into the environment. Most importantly, the U.S. Department of Agriculture has the greatest supply of raw materials from which to isolate, characterize, and manipulate these extremely potent regulatory molecules, which are present in nature in such infinitesimally small quanities.

Resources Needed in ARS in Peptide Research

Initially basic equipment needs were extensively discussed and the requirement for adequate support at individual laboratory level was emphasized. The consensus among the participants was that the laboratories involved are underfunded by at least 25%.

Recommendations were to strengthen this program by:

-- Research support of the basic efforts at the SY level in peptide research, i.e., insect physiology, cellular biochemistry, cellular physiology, peptide chemistry, peptide biochemistry, molecular genetics, and neuroendocrimology

-- Increase available technical support. This research area is in a high technology field that requires sophisticated analytical instrumentation that demands considerable time from the involved research scientists. ARS has a need for skilled support scientists in this role as well as qualified technicians for biologically oriented procedures. Postdoctoral and graduate student programs could be utilized within these program areas.

-- Retain the commitment to develop the College Station Facility as a center for peptide sequencing and peptide synthesis for ARS researchers.

-- Consider future equipment and procedures necessary for developing pest management strategies in coordination with the basic peptide research programs.

-- Retain emphasis on communications and exchange of ideas and preliminary results with continuing informal workshops.

-- Noted that plant peptide researchers also need further representation with peptide research area.

Cooperation and Coordination in the ARS

-- Recommended organization of a Peptide Technical Service Group. Group should include one representative each from the following areas: plant peptides, vertebrate neuropeptides, invertebrate neuropeptides, and peptide chemistry.

The members of the technical service group would serve individually and/or together to:

a. Serve as a focal point for communication among ARS groups working with peptides.

b. To serve as a technical resource to provide information and recommendations to working ARS groups.

c. To help in organization of workshops.

d. To serve as liaison between the NPS and the working groups.

e. To identify research needs and opportunites and communicate them to the NPS.

f. To alert working groups to opportunities for grants and in-house funding.

-- The group also recommended the compilation of a catolog of ARS personnel, capabilities and facilities, and specialized equipment. This catalog to be distributed to all ARS labs working with peptides to facilitate interaction.

Opportunities for New Research on Peptides in ARS

OBJECTIVES:

	A.	B.	C.
Insect	Pest control through (a) Direct application of modified-synthetic peptides (b) Use of inhibitors of production, release or action of peptides	Enhanced use of beneficial insects through use of peptides in the artificial rearing of important beneficial insects	Improved mass rearing for sterile release programs through use of peptide regulators of diapause, behavior, growth and metamorphosis
Vertebrate	Improved reproductive efficiency in farm animals through use of peptide regulators	Improved efficiency of conversion of domestic farm animal and poultry food into human food through use of peptide growth factors	Reduced requirements of animals for food suitable for people through use of regulatory peptides that affect food utilization
Plant	Enhance fundamental processes such as plant growth and ripening of fruit through use of peptides and related molecules	Utilize plants to produce peptides for pharmaceutical applications	Utilize mycotoxins to control weeds and other unwanted plant growth, or mycotoxin antagonists to protect crop plants.

Opportunities for New Research on Peptides in ARS

RESEARCH NEEDED:

	A.	B.	C.
Insect	(a) Isolate and identify peptides that control basic processes of growth, reproduction, behavior, and homeostasis. (b) Improve understanding of neuroanatomy and ultrastructure of neurosecretory systems. (c) Improve capability for tissue culture bioassays. (d) Enhance capability in biochemistry and and molecular genetics of peptide research.	(a) Isolate and identify peptides and related molecules that are critical to artificial rearing of beneficial insects	(a) Isolate and identify peptide regulators of growth and diapause that may be important for efficient mass rearing of key species (b) Investigate potential for gene insertion to promote desired parameters in mass reared species.
Vertebrate	Describe and manipulate the mode of genomic expression and post-translational modification of specific peptides that control reproduction, growth and digestion in farm animals.		
Plant	Isolate, identify, and synthesize peptide regulators of plant growth and ripening	Identify the presence of putative pharmaceuticals produced by plants, such as cyclosporins	Isolate and identify additional mycotoxins and develop antagonists to them

Representative Research Sequence

1. Identify the bioactivity of a putative peptide regulator
2. Develop bioassay
3. Isolate and purify the peptide
4. Investigate the site of production, mode of release; transport and site of action of the peptide.
5. Investigate cellular and genomic action
6. Investigate biosynthesis, post-translational modifications, and relate to DNA sequences
7. Synthesize subject peptide and analogues
8. Investigate potential for production through gene cloning techniques
9. Develop strategies for use

PROGRAM

BIOLOGICALLY ACTIVE PEPTIDE RESEARCH WORKSHOP

Beltsville Agricultural Research Center-EAST
Conference Room, Building 177B

Wednesday, October 31, 1984

A.M.	8:30 - 8:45	Welcome and Introductions - Borkovec, A. B. Wright, J. E.
	8:45 - 9:00	Holman, M. Peptide research at VTERL.
	9:05 - 9:20	Cook, B. J., and Meola, S. The dorsal diaphragm: A neurohemal organ in the stable fly.
	9:25 - 9:35	Meola, S. Necessity of comparative structural studies as a basis for neuroendoctrine approach to insect control.
	9:40 - 10:00	Wagner, R. M. Sequencing of neuropeptides.
	10:00 - 10:30	Break
	10:30 - 10:45	Masler, E. P. Neurochemical factors and ovarian development in the mosquito.
	10:50 - 11:00	Kelly, T. J. Biological specificity of Manduca PTTH and Aedes EDNH.
	11:05 - 11:20	Loeb, M. J. Pupal brain extracts induce immature testes to secrete ecdysteroids.
	11:25 - 11:40	Gelman, D. B. Head factors influencing eupyrene and apyrene spermatogenesis in the European corn borer.
	11:45 - 11:55	Hayes, D. K.
P.M.	12:00 - 1:00	Lunch

	1:00 - 1:20	Jaffe, H. Recent progress on the isolation, purification, and identification of insect neuropeptides.
	1:25 - 1:40	Bolt, D. J. Isolation and purification of inhibin, a polypeptide from the ovary.
	1:45 - 2:00	Lunney, J. Importance of immune response genes in the regulation of immune response to peptides.
	2:05 - 2:25	Kraeling, R. R. Peptide regulation of reproduction and growth and development in the pig.
	2:30 - 2:45	Moore, D. M. Mapping neutralizing epitopes of foot-and-mouth disease virus with monoclonal antibodies and synthetic peptides.
	2:50 - 4:30	Visits of BARC laboratories.

Thursday, November 1, 1984

A.M.	8:30 - 12:00	Research planning and discussion: ARS research on peptides and related subjects. J. E. Wright
P.M.	12:00 - 1:00	Lunch
	1:00 - 4:30	Visits of BARC laboratories.

List of Attendees and Addresses

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South Atlantic Area

3. Number and title of CRIS work unit:

7602-20250-007 Regulation of Insect Growth and Development
7602-20620-13 Physiology of Stored Product Insects

4. Approach Element and Problem Definitions:

2.4.01.1.c Differentiation and Morphogenesis of Insect Cells
2.4.01.1.d Insect Exoskeleton
4.3.01.1.a Physiology and Biochemistry of Stored Product Insects

5. Estimated SY's:

1.0

6. Objectives of research:

To develop a fundamental understanding of insect macromolecules unique to insect systems that will provice a basis for new concepts in selective and environmentally safe control of harmful insects and improvement of beneficial insects.

7. Research priorities in your program:

Develop information on the hormonal regulation of macromolecules that function as key nutrients, structural components, and regulators of insect growth and development.

Harmful Insects--Attention is given to possible unique features in the structure of the molecules and in their mode of biosynthesis for comparison with other organisms as a basis for the development of selective pesticides.

Beneficial Insects--Focus is on the importance of regulatory molecules in the embryonic development of insect endoparasitoids. This research determines the physiological mechanisms that regulate uptake of materials by the parasitoid embryo from the host insect's hemolymph and the synthesis of macromolecules by the embryo.

8. Progress of current research in solving problems:

(a) Currently isolating and purifying a protein macromolecule that induces cellular morphogenesis in an insect cell line.

(b) Identified proteins synthesized at different stages of development of the parasitoid embryo and now am identifying the mechanism(s) that regulate synthesis of the macromolecules by the embryo and uptake of materials by the parasitoid embryo from the host insect's hemolymph.

9. Significant research accomplishments in the past 3 years:

(a) I developed a novel microtechnique for measurement of chitin biosynthesis in isolated tissues. This research is important in that it paved the way for investigation of hormone-induced chitin synthesis in tissue culture. The study of chitin synthesis is important in that chitin is an important constituent of the cuticle and is the target for certain inhibitors and putative insectides.

(b) Demonstrated that pheromone-hydrolyzing esterases are present on the external surfaces of the legs and other body parts of adult moths. The antennae of newly emerged adult moths were found to acquire the capacity to hydrolyze the pheromone when they are also able to respond to the pheromone. This research is important because it provides data for the first time that supports the hypothesis that enzymes capable of degrading a pheromone may play a dual role in olfaction on the antenna and other cuticular body surfaces to prevent build-up of pheromone molecules in these regions which would otherwise interfere with the insect's chemical communication system.

(c) I developed a new research program on the biochemistry of parasitoid development and demonstrated that a newly deposited parasitoid egg has the protein synthesis machinery necessary for development of the embryo, and that host hemolymph proteins probably have a regulating

. . .

effect on the egg's metabolism rather than a nutritional role. The research is important in that it paves the way for subsequent studies on the regulation of protein synthesis at the genetic level. In addition, the basic research on host hemolymph proteins will aid other investigators to imitate in culture media the protein composition of the host's hemolymph in the development of an artificial rearing medium for the parasitoids.

(d) I isolated a proteinaceous fraction from insect hemolymph that induces the formation of vesicles in an insect cell line. This research is important in that it keys in on a protein regulatory molecule that directs the formation of a cellular structure in an orderly manner.

10. Impact of research accomplishments on science and the general public:

The research has a high potential for opening up new areas of insect biochemistry that could lead to breakthroughs in pest control technology. It is important to the public in that the new selective insect control technologies that will be derived from this research will be used to reduce the loss of food and fiber to insect pests while at the same time protect the environment and non-target organisms.

11. Obstacles to achieving objectives:

Little is known about the identity and hormonal control of regulatory macromolecules that may be unique to insects; therefore, fundamental background information is unavailable. Also, the literature available on the biochemistry and molecular biology of embryogenesis is lacking. Additionally, the small size of the wasp embryos present an obstacle in acquiring adequate amounts of biological material for biochemical analyses.

12. Future lines of needed research and plan for implementation:

Plans are to identify and isolate key regulatory macromolecules important at the cellular level in insect and growth and development and in embryogenesis of endoparasitoids and subsequently to investigate the biochemical mechanisms responsible for their biosynthesis.

13. Research facilities and personnel needs:

I need a HPLC instrument for purification of the protein molecules and additional laboratory space plus technical help.

14. Extent of cooperation--names of persons and institutions:

15. Titles of publications for the last 3 years:

Ferkovich, S. M. Enzymatic alteration of insect pheromones. In Dale M. Norris (ed), Perception of Behavioural Chemicals, pp. 165-185. Elsevier/ North-Holland Biomedical Press, Amsterdam. 1981. (Book Chapter)

Ferkovich, S. M., Oberlander, H., and Leach, C. E. Chitin synthesis in larval and pupal epidermis of the Indian meal moth, Plodia interpunctella (Hübner), and the greater wax moth, Galleria mellonella (L.). J. Insect Physiol. 27(8):509-514. 1981.

Taylor, T. R., Ferkovich, S. M., and Van Essen, F. Increased pheromone catabolism by antennal esterases after adult eclosion of the cabbage looper moth. Experientia 37:729-731. 1981.

Ferkovich, S. M., Oliver, J., and Dillard, C. Comparison of pheromone hydrolysis by the antennae with other tissues after adult eclosion in the cabbage looper moth, Trichoplusia ni. Ent. exp. & appl. 31:327-328. 1982.

Ferkovich, S. M., Oliver, J. E., and Dillard, C. Pheromone hydrolysis by cuticular and interior esterases of the antennae, legs, and wings of the cabbage looper moth, Trichoplusia ni (Hübner). J. Chem. Ecol. 8(5): 859-866. 1982.

Ferkovich, S. M., Greany, P. D., and Dillard, C. Changes in haemolymph proteins of the fall armyworm, Spodoptera frugiperda (J. E. Smith), associated with the parasitoid, Apanteles marginiventris (Cresson). J. Insect Physiol. 29:933-942. 1983.

Greany, P. D., Ferkovich, S. M., and Hanrahan, A. M. Use of dialyzed, concentrated fetal bovine serum as a medium supplement for the endoparasitoid Apanteles marginiventris (Cresson). XI International Conference on Invertebrate Tissue Culture, St. Augustine, FL, June 1983. (Abstract)

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3. Number and title of CRIS work unit:

7602-20250-007
Regulation of Insect Growth and Development

4. Approach Element and Problem Definitions:

2.4.01.1.e / Nutrition and rearing requirements of beneficial organisms.

5. Estimated SY's:

1 SY

6. Objectives of research:

Define nutritional and other physiological requirements of parasitic insects toward development of in vitro culture systems.

7. Research priorities in your program:

To elucidate the role of host-produced biochemicals as nutrients and/or hormones in the growth and development of a parasitic wasp, Microplitis croceipes.

8. Progress of current research in solving problems:

Recent studies on early embryogenesis of M. croceipes showed that a high molecular weight component, most likely a polypeptide, of host hemolymph is absolutely required for normal development to occur. Current studies are directed toward isolation and identification of the factor(s) responsible. In addition, insect hormones (including a peptide hormone) are being evaluated in relation to their potential influence on growth and molting of parasite larvae cultured in vitro.

9. Significant research accomplishments in the past 3 years:

Development of in vitro culture methods that have enabled basic biochemical and nutritional studies on insect parasites, includin two species of hymenopterous larval endoparasites that attack the fall armyworm and the corn earworm, respectively, plus a dipteran endoparasite of the gypsy moth.

In addition to this research, with the aid of coworkers, we have partially elucidated the mechanisms by which citrus fruit resist fruit fly attack and have pioneered a new technology involving us of a plant growth regulator to sustain the resistance of the frui and reduce its susceptibility to attack.

10. Impact of research accomplishments on science and the general public:

This research could have a significant influence on insect pest control as it could enable, for the first time, the mass production of beneficial insect parasites at low cost. This in turn would enhance their use as biorational pest control agents, with commensurate ecological and economic benefits.

11. Obstacles to achieving objectives:

Lack of sufficient knowledge about the basic biology of the organisms being studied; i.e., there is an insufficient knowledge base concerning the fundamental nutritional physiology of insect parasites, especially larval endoparasites. This is in turn a reflection of a relative lack of funding over past years for research on the basic biology of these organisms.

12. Future lines of needed research and plan for implementation:

Significantly more research on the basic nutritional and endocrin physiology of the endoparasites being studied will be needed before it will be possible to successfully rear them in vitro. Analytical biochemical studies are needed, and are being pursued.

13. Research facilities and personnel needs:

The facilities at this Laboratory are appropriate and adequate fo the work in question. However, progress would be accelerated if suitably trained personnel (i.e., a biochemically-competent postdoctoral) were available to assist in gaining the needed background information.

14. Extent of cooperation--names of persons and institutions:

Dr. Drion Boucias of the University of Florida Dept. of Entomolog is providing limited assistance in performing gel electrophoresis and biochemical fractionation studies ancillary to the in vitro culture work. Dr. Steven Ferkovich, also of the IABBBRL, is performing biochemical studies relevant to my own nutritional research on M. croceipes.

15. Titles of publications for the last 3 years:

Greany, P. D. Culture of hymenopteran endoparasitoids in vitro. In Vitro 17: 230. 1981. (Abstract)

Greany, P. D., and Hagen, K. S. Prey selection, pp. 121-135. In: Nordlund, D. L., Jones, R. L., and Lewis, W. J. (eds.), Semiochemicals: Their Role in Pest Control. J. Wiley and Sons, New York. 306 pp. 1981.

Greany, P. D., Burditt, A. K., Jr., and Chambers, D. L. 1982. Effectiveness of Jackson traps for fruit flies improved by addition of colored patterns. Fla. Entomol. 65:374-375.

Greany, P. D., S. C. Styer, P. L. Davis, P. E. Shaw, and D. L. Chambers. 1983. Biochemical resistance of citrus to fruit flies. Demonstration and elucidation of resistance to the Caribbean fruit fly, Anastrepha suspensa. Ent. exp. & appl. 34: 40-50.

Styer, S. C., and P. D. Greany. 1983. Increased susceptibility of laboratory-reared vs. wild Caribbean fruit fly, Anastrepha suspensa (Loew) (Diptera: Tephritidae), larvae to toxic citrus allelochemics. Environ. Entomol. 12: 1606-08.

Burditt, A. K., Jr., T. P. McGovern, and P. D. Greany. 1983. Anastrepha suspensa (Loew) (Diptera: Tephritidae) response to chemical attractants in the field. Proc. Fla. State Hort. Soc. 96: 222-226.

Ferkovich, S. M., P. D. Greany, and C. Dillard. 1983. Changes in haemolymph proteins of the fall armyworm, Spodoptera frugiperda (J. E. Smith), associated with parasitism by the braconid parasitoid Apanteles marginiventris (Cresson). J. Insect Physiol. 29: 933-942.

King, E. G., A. Baumhover, L. F. Bouse, P. Greany, A. W. Hartstack, K. R. Hopper, E. F. Knipling, R. K. Morrison, W. C. Nettles, and J. E. Powell. Control of Heliothis spp. by augmentation of entomophagous arthropods. For: Theory and Tactics of Heliothis Population Management: I. Cultural and Biological Control (Proceedings of S-59 Workshop), S. Johnson and J. R. Bradley (eds.) (in press).

Greany, P. D., S. B. Vinson, and W. J. Lewis. Insect parasitoids: Finding new opportunities for biological control. BioScience (in press for December 1984 issue).

Greany, P. D., P. E. Shaw, P. L. Davis, and T. T. Hatton. Senescence-related susceptibility of Marsh grapefruit to laboratory infestation by Anastrepha suspensa (Diptera: Tephritidae). Fla. Entomol. (in press).

Greany, P. D. Growth and development of two hymenopterous endoparasitids in vitro. J. Insect Physiol. (in press).

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3. Number and title of CRIS work unit:

7602-20250-018 Genetics of Sterility in *Heliothis*

4. Approach Element and Problem Definitions:

2.2.01.1.f Insect Genome Organization
2.2.01.1.g Insect Gene Expression and Regulation
2.4.01.1.c Differentiation and Morphogenesis

5. Estimated SYs:

1.0

6. Objectives of research:

This research program has been designed to probe fundamental aspects of the differentiation and development of insect spermatozoa. The core objective of this work is to elucidate the molecular basis of male sterility in *Heliothis virescens* x *H. subflexa* backcross hybrids. The gene or genes which give rise to this phenomenon will be isolated, characterized, and manipulated such that male sterility can be genetically engineered into other pest species.

7. Research priorities in your program:

(1) Document important events during the development of sperm from fertile and sterile testes.

(2) Clone genes regulating these events and determine their structure(s) and function(s) in fertile and sterile individuals.

(3) Introduce these cloned genes into insect cell lines using expression vectors in order to determine precise functions of gene products (polypeptides).

(4) Develop the means to transform lepidopteran germlines with defined genes which may result in the sterilization of male transformants.

8. Progress of current research in solving problems:

(1) Sperm mitochondrial proteins have been isolated, antisera are being prepared for detailed studies.

(2) Genomic libraries have been established for H. virescens, H. subflexa, and H. armigera.

(3) cDNA libraries from H. virescens testes, embryos, and fat body have been established.

9. Significant research accomplishments in the past 3 years:

(1) The entire complement of polypeptides synthesized during sperm development has been documented.

(2) Sperm mitochondrial proteins (both cytoplasmic and organellar) have been identified.

(3) A species-specific mitochondrial protein has been implicated in causing sterility (purified, currently raising antiserum).

(4) Clones corresponding to nine unique mitochondrial genes have been isolated and characterized.

(5) Steady-state levels of mitochondrial RNAs have been compared between fertile and sterile testes--levels in the latter have been shown to be 3- to 10-fold lower.

(6) α- and ß-tubulins from sperm have been isolated and developmental profiles determined--isolation of corresponding genes is underway.

10. Impact of research accomplishments on science and the general public:

Progress in this area of research begins to unravel the complexities of the development of insect spermatozoa. In particular, the availability of cloned defined genes which regulate spermatogenesis will, in the short-term, allow this important developmental process to be manipulated in the laboratory.

Ultimately, this work may allow scientists to sterilize insects in rearing facilities without resorting to irradiation. Mass-release programs utilizing these lines should prove to be useful in pest insect population suppression.

11. Obstacles to achieving objectives:

(1) Failure to develop a simple, reliable, and precise means of transforming germlines.

(2) Inability to design gene regulatory signals which are sensitive to desired environmental cues.

12. Future lines of needed research and plan for implementation:

The work of several laboratories collaborating on germline transformation techniques will be required to meet the above objectives. Collaboration with Drosophila geneticists may be a practical approach since success has already been achieved in this area.

Input from population biologists/geneticists will also be necessary in order to design workable release programs. In particular, models documenting gene flow and allele frequency changes need to be constructed for each scheme.

13. Research facilities and personnel needs:

(1) 2-3 additional laboratories/SYs devoted to gene manipulation in insects.

(2) Funds for postdoctoral support in these efforts.

14. Extent of cooperation--names of persons and institutions:

This program currently receives no input from outside collaborators. The construction of the first cDNA library was performed in the laboratory of Dr. Lee Weber (University of South Florida, Tampa, FL). Input on the genetic aspects of the sterility phenomenon was provided by Dr. Milton Huettel (formerly at this location).

15. Titles of publications for the last 3 years:

Miller, S., and Huettel, M. An analysis of sperm proteins in Heliothis virescens, H. subflexa, and their sterile backcross progeny. (Manuscript in preparation)

Miller, S., Weber, L., Weber, E., and Huettel, M. Patterns of mitochondrial DNA transcription in testes of the Heliothis spp. complex. (Manuscript in preparation)

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3. Number and title of CRIS work unit:

7602-20250-007 Regulation of Insect Growth and Development
7602-20620-13 Physiology of Stored Product Insects

4. Approach Element and Problem Definitions:

2.4.01.1.c Differentiation and Morphogenesis of Insect Cells
2.4.01.1.d Insect Exoskeleton
4.3.01.1.a Physiology and Biochemistry of Stored Product Insects

5. Estimated SY's:

1.0

6. Objectives of research:

To provide the basis for biorational, environmentally safe novel methods of controlling insect pests through interference with the processes of insect growth and development and their neuroendocrine control.

7. Research priorities in your program

Utilize cell lines to identify and determine the action of new regulatory molecules:

(a) Establish hormonally responsive cell lines in vitro

(b) Develop cell lines that undergo morphogenesis in response to regulatory molecules

(c) Develop cell lines that produce chitin in vitro

8. Progress of current research in solving problems:

(a) 3 cell lines derived from lepidopteran imaginal discs have been established

(b) 2 cell lines drastically alter their morphogenetic properties in response to a newly discovered macromolecular factor

(c) 1 cell line responds to hormones with increased uptake of chitin precursors, and may produce chitin

9. Significant research accomplishments in the past 3 years:

I developed a detailed and novel analysis of chitin biosynthesis in tissues stimulated with ecdysteroids. Chitin is not only the major unique constituent of cuticle, but is a likely target for certain insect growth regulators that have potential for pest control. The research showed that the initiation of chitin synthesis in response to 20-hydroxy-ecdysone required RNA and protein synthesis. I extended my findings from imaginal discs to larval tissues and proposed an overall model for the hormonal control of chitin synthesis in insects.

I contributed to a new area of research, the effects of insect hormones on cell lines. In the first phase, clones were made from embryonic Manduca cell lines. These clones responded to ecdysteroids by changing cellular shape as a result of protein-synthesis dependent changes in the cytoskeleton. Subsequently, cell lines were derived from imaginal discs. These are the first cell lines from a specific insect tissue that maintain their responsiveness to hormones. This pioneering work should stimulate considerable activity in the study of hormonal action in cell lines of known tissue origin.

10. Impact of research accomplishments on science and the general public:

The isolation, identification and mode of action of novel regulatory molecules that orchestrate insect development will lead to new perspectives for the control of insect pests in a manner that is target-specific, effective and environmentally safe.

11. Obstacles to achieving objectives:

Necessary tissue culture bioassays are still limiting.

Protein purification methods need to be improved to isolate regulatory molecules from tissue culture systems.

12. Future lines of needed research and plan for implementation:

Additional cell lines from tissue of known origin need to be established.

Collaborative research between tissue culturists and biochemists needs to be strengthened to successfully utilize invitro systems for the discovery of new regulatory molecules.

13. Research facilities and personnel needs:

(1) Modern HPLC equipment for purification of regulatory molecules

(2) Postdoctoral scientist for biochemical work

14. Extent of cooperation--names of persons and institutions:

Cooperative Research (outside of Gainesville)
Dr. Dwight Lynn, Insect Pathology Laboratory, Beltsville

Cell lines provided as requested to government and university laboratories:

15. Titles of publications for the last 3 years:

Lynn, D. E. and H. Oberlander. The effect of cytoskeletal disrupting agents on the morphological response of cloned Manduca sexta cell line to 20-hydroxy-ecdysone. Wilhelm Roux's Archives of Developmental Biology 190:150-155. 1981.

Oberlander, H., C. E. Leach, and D. E. Lynn. Effects of cycloheximide on cellular elongation in a Manduca sexta cell line. Wilhelm Roux's Archives of Developmental Biology 190:60-61. 1981.

Ferkovich, S. M., H. Oberlander, and C. E. Leach. Chitin synthesis in larvl and pupal epidermis of the Indian meal moth, Plodia interpunctella (Hübner), and the greater wax moth, Galleria mellonella (L.). J. Insect Physiol. 27:509-514. 1981.

Miller, S. G. and H. Oberlander. Identification of newly synthesize wing imaginal disc proteins induced by 20-hydroxyecdysone in Galleria mellonella. In Vitro 17:689-694. 1981.

Oberlander, H. and D. E. Lynn. Morphogenesis in insect tissue culture. In Advances in Cell Culture, Vol. II. K. Maramorosch, Ed. pp. 237-265. 1982.

Oberlander, H. Imaginal discs. In Endocrinology of Insects. R. Downer and H. Laufer, Eds. pp. 503-507, Alan R. Liss Inc., NY, 1983.

Lynn, D. E., S. G. Miller and H. Oberlander. Establishment of a cell line from lepidopteran wing imaginal discs: Induction of newly synthesized proteins by 20-hydroxyecdysone. Proc. National Academy of Science (USA) 79:2589-2593. 1982.

Oberlander, H., D. E. Lynn and C. E. Leach. Inhibition of cuticle production in imaginal discs of Plodia interpunctella (cultured in vitro): Effects of colcemid and vinblastine. J. Insect Physiol. 29:47-53. 1983.

Lynn, D. E. and H. Oberlander. The establishment of cell lines from imaginal wing discs of Spodoptera frugiperda and Plodia interpunctella. J. Insect Physiol. 29:591-596. 1983.

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3. Number and title of CRIS work unit:

7602-20620-012 (50%) Reproductive Behavior of Stored-Product Insects
7602-20620-014 (50%) Behavioral Ecology, Manipulation and Control of Insect Pests of Stored Products

4. Approach Element and Problem Definitions:

4.3.01.1.a Physiology and Biochemistry of Stored Product Insects

5. Estimated SY's:

1.0

6. Objectives of research:

To examine the hormonal mechanisms regulating the production of yolk polypeptides and the formation of yolk during egg maturation in the stored product pest, Plodia interpunctella, and the subsequent utilization of the yolk components by the growing embryo during embryogenesis. This basic information will be applied to formulate programs to manipulate the reproductive capacity of these insects.

7. Research priorities in your program

A) To identify the hormonal components regulating production of the yolk polypeptides and how these components are coordinated with the completion of pharate adult development

B) To obtain DNA clones to the yolk polypeptide genes which will be examined for structural features necessary for hormonal activation of the genes and will be used to identify tissue specific sites of yolk polypeptide production as well as to quantitate messenger RNA production.

C) To identify the mechanisms directing the utilization of specific yolk components during embryogenesis.

8. Progress of current research in solving problems:

We have found four major yolk polypeptides (YPs) in mature eggs of Plodia interpunctella and have named them by decreasing molecular weight: YP1=153,000 daltons, YP2=70,000 daltons, YP3=46,000 daltons and YP4=33,000 daltons. By in vivo labeling and incubation of organs in vitro, YP1 and YP3 were shown to be secreted by the fat body into the hemolymph and transported to the eggs for uptake. Thus YP1 and YP3 can be considered to be vitellogenins. Similarly, YP2 and YP4 were shown to be produced within the ovaries and taken up by the eggs without entering the hemolymph. All four YPs were shown to be unique, unrelated polypeptides by peptide mapping. Within the egg, YP1 and YP3 associated as a single protein, and YP2 and YP4 associated as a protein. These associations were determined by the techniques of immunodiffusion, gel permeation chromatography, pore exclusion native gel electrophoresis, and sedimentation by ultracentrifugation. Poly(A) RNA was isolated from ovaries and translated in a cell-free reticulocyte lysate. Examination of the products produced by the translation showed YP2 and YP4 were found to be the products of two separate mRNAs. Vitellogenic processes begin about halfway through pharate adult development, and are maximal at adult eclosion. Treatment of pharate adult females with the hormone 20-hydroxyecdysone blocked egg maturation and specifically the synthesis of the YPs. After fertilization of normal eggs, we found that embryos less than 48 hours old were specifically digesting YP2 from the yolk complex and that the other three YPs were used during the remainder of embrogenesis and early egg larval period. From this work, we have identified the major yolk polypeptides, where they are produced, what associations they form, a hormone that may be involved in the control of their production, and which are utilized during embryogenesis.

9. Significant research accomplishments in the past 3 years:

1) This investigation is the first to examine yolk production and utilization in a lepidopterous stored products pest.

2) The YPs differ in molecular sizes from those found in other lepidopterans.

3) The ovaries produce two major YPs packaged in the eggs.

4) The YPs produced in the ovaries associate as a protein in the egg.

5) The control of the vitellogenic processes and specifically the synthesis of the YPs is dependent upon the withdrawal of the hormone 20-hydroxyecdysone (20HE), and that addition of 20HE blocks the normal progression of vitellogenesis during pharate adult development.

10. Impact of research accomplishments on science and the general public:

1) This is the first analysis of the control of yolk production in the pyralid moths. From a comparative physiology aspect, the results have shown Plodia to have some characteristics in common with the other lepidopterans, but that there are unique differences which make the continued analysis worthwhile. The most important of these differences appears to be in production of two YPs in the ovary that constitute a major protein of the yolk that has a primary role in supplying nutrients during embyrogenesis.

2) This study suggests that the development of the pharate adult during metamorphosis is coordinated by the decreasing titres of 20HE, and that 20HE has an inhibitory action on the production of the yolk for egg maturation. This is the first case where the inhibitory action of 20HE can be demonstrated on a specific gene product.

3) The impact on the general public comes from the perspective that we know metamorphosis and egg production can be disrupted in these insects by disturbing the normal hormonal program during adult development. Further examination of the endocrine mechanisms may allow us to derive compounds that will act to effectively block the completion of egg maturation and effect control of this stored product pest.

11. Obstacles to achieving objectives:

The major obstacle to understanding the nature of the endocrine mechanisms controlling the vitellogenic process in Plodia will be determining whether the hormone 20-hydroxyecdysone is acting directly on the genes or manifests its action on a secondary level. The analysis of this problem is complicated by the nature of the response to 20HE which is inhibitory to the YP genes. This means that when the hormone is administered, the product we are measuring, that is the YP gene products, is diminishing. Therefore, we need the most sensitive measures of the YP gene activity, which at this time would be the use of cloned DNA containing the YP genes.

12. Future lines of needed research and plan for implementation:

To be able to further examine the nature of the hormonal action on the YP genes it will be necessary to obtain cloned YP gene DNA. This will be approached from two experimental lines (1) deriving the clones by searching a gemonic DNA library with complementary DNA made to RNA from either the fat body or the ovaries and 2) searching a DNA library with a DNA probe synthesized from the amino acid sequence of a unique peptide fragment from each of the YPs purified by high pressure liquid chromatography. Once obtained, the clones can then be used to determine whether the 20HE is acting at a primary or secondary level to effect control of the YP genes.

13. Research facilities and personnel needs:

During the next three years, it will be necessary to have the assistance of one laboratory technician who is competent in gel electrophoresis and general biochemical techniques. In addition, one postdoctoral associate will be necessary to assist in cloning the genes for the YPs and implementing their use in examining the control of transcription of these genes.

Major equipment needed for this work will be a controlled temperature growth chamber for growing bacteria, high voltage electrophoresis equipment for DNA sequencing, computer linked gel scanner for quantitative analysis of protein and transcript production, computer based system for DNA sequence analysis and HPLC with computer assisted UV detector for identification and purification of peptide fragments.

14. Extent of cooperation--names of persons and institutions:

Daniel Bean, Oregon State University, Corvallis, Oregon
Victor J. Brookes, Oregon State University, Corvallis, Oregon

15. Titles of publications for the last 3 years:

Shirk, P. D., D. Bean, A. M. Millemann, and V. J. Bookes. Identification, synthesis and characterization of the yolk polypeptides of Plodia interpunctella. J. Exp. Zool. 232:87-98. 1984

Shirk, P. D., G. Bhaskaran, and H. Röller. Developmental Physiology of corpora allata and accessory sex glands in the cecropia silkmoth. J. Exp. Zool. 227:69-79. 1983.

Shirk, P. D., P. Minoo and J. H. Postlethwait. 20-Hydroxyecdysone stimulates the accumulation of translatable yolk polypeptide gene transcript in adult male Drosophila melanogaster. Proc. Natl. Acad. Sci. USA, 80:186-190. 1983

Dahm, K. H., G. Bhaskaran, M. G. Peter, P. D. Shirk, K. R. Seshan, and H. Röller. The juvenile hormones of cecropia. In Regulation of Insect Development and Behavior. (F. Sehnal, A. Zabza, J. J. Menn, and B. Cymborowski, eds.), pp. 183-198, Technical University of Warsaw, Warsaw, Poland. 1981

Peter, M. G., P. D. Shirk, K. H. Dahm, and H. Röller. On the specificity of juvenile hormone biosynthesis on the male cecropia moth. Z. Naturforsch. 36c:579-585. 1981

Postlethwait, J. H. and P. D. Shirk. Genetic and endocrine regulation of vitellogenesis in Drosophila., Amer. Zool. 21:687-700. 1981

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3. Number and title of CRIS work unit:

7602-20251-001 Mechanism of Protein Transport through Cell Membranes and its Hormonal Control

7602-20620-013 Physiology of Stored Product Insects

4. Approach Element and Problem Definitions:

2.4.01.1.a Membrane Transport
2.4.01.2.a Physiology and Biochemistry of Stored Product Insects

5. Estimated SY's:

1.0

6. Objectives of research:

(1) To determine the function of storage proteins in insect development.
(2) To determine the mechanism of hormonal regulation of storage protein synthesis during insect development.
(3) To determine the structural features of storage proteins that are instrumental for their transport through cell membranes.
(4) To determine the mechanism of hormonal regulation of storage protein transport through cell membranes.

7. Research priorities in your program:

My program has two primary research objectives:

(1) To identify hormones responsible for initiating metabolic changes in larvae that are prerequisite for metamorphosis to the adult; JH and ecdysone do not appear to be solely responsible.

(2) To develop a tissue-specific delivery system for biologically active molecules and/or liposomes based on those molecular characteristics functioning in receptor-mediated endocytotic uptake processes.

Achieving these objectives in the short term (i.e., 3-5 years) will indicate the feasibility of applying this technology for the control of pest insects. In-depth studies on the biochemical mechanisms will be phased into these experiments when the feasibility of these approaches is established.

8. Progress of current research in solving problems:

In the past three years we have successfully completed pilot scale testing of the most effective IGRs for the control of moths infesting stored peanuts. The peanut industry is eagerly awaiting the final registration and approval for use of this methodology on edible commodities.

Our new focus has turned to identifying biochemical events initiated in larvae that are preparatory for metaborphosis to the adult. Most conspicuous are the accelerated syntheses of lipoproteins, storage proteins, and silk proteins during the last larval instar. We are currently involved in identifying the hormonal factors that regulate the syntheses and transport of these proteins.

Concurrently, we have been examining the structural features of storage proteins that are recognized by receptors mediating their uptake and storage in the fat body. We have determined that waxmoth storage protein consists of three identical protein subunits. Each subunit has a single covalently linked glycan of the high-mannose type. We are now beginning studies to determine the role of the glycan in the uptake process.

9. Significant research accomplishments in the past 3 years:

The culmination of ten years of basic investigations on JH and IGR effects on insect growth and development was realized with the successful application of these chemicals to commodities stored under warehouse conditions.

Storage proteins have been identified, isolated, and partially characterized from waxmoths at different stages of development. Lipoproteins in waxmoths have been identified and quantified at different developmental stages. These two systems are now adequately defined for pursuance of current investigations on the hormonal regulation of synthesis and transport of these special developmental proteins.

10. Impact of research accomplishments on science and the general public:

The use of IGRs on stored peanuts for control of stored product pests is highly significant because traditional control procedures are now prohibited or have lost their effectiveness.

The impact of my current studies will be realized within the next 3-5 years. The development of the new technologies requires the ability to deliver it to pest populations in the field. My studies should provide a delivery system for a variety of biologically active molecules (e.g., genes, neurohormones, toxins, etc.), targeting them to specific tissues within the insect pest.

11. Obstacles to achieving objectives:

One major difficulty has been the discontinuity that yearly funding cycles bring about. Too often moneys are not available until the last half of the year and as a consequence can't be used as effectively as they might be. If projects were funded for three-year periods, it would make hiring of high quality postdoctorals and technicians much easier and provide the stability necessary for the focused, highly intense investigations required in today's research.

12. Future lines of needed research and plan for implementation:

In-depth studies are planned for investigating the biochemical mechanisms of the hormonally regulated uptake and utilization of storage proteins. Additionally, studies on the mechanisms of protein glycosylation and processing are planned when additional personnel are available. The ultimate objective will be to develop highly specific delivery systems for bioregulatory molecules.

13. Research facilities and personnel needs:

(1) Modern instrumentation is sorely needed for isolation of biologically active molecules that function at very low levels in the insect.

(2) A Postdoctoral scientist is needed to conduct studies on protein glycosylation.

14. Extent of cooperation--names of persons and institutions:

In the past I have had cooperative research projects with Dr. Avram Friedlander of the Volcani Center in Israel. Future plans are to establish a new project with Dr. Eli Schaaya of the same institution.

Informal cooperation with Dr. Krishna Kumaran of Marquette University and Dr. Leo Levenbook of NIH has begun. I intend to conduct more formal cooperation in the future.

15. Titles of publications for the last 3 years:

Miller, S. G. and Silhacek, D. L. Identification and purification of storage proteins in tissues of the greater wax moth, Galleria mellonella (L.). Insect Biochem. 12(3):277-292. 1982.

Miller, S. G. and Silhacek, D. L. The synthesis and uptake of haemolymph storage proteins by the fat body of the greater wax moth, Galleria mellonella (L.). Insect Biochem. 12(3):293-300. 1982.

Miller, S. G. and Silhacek, D. L. The effects of tunicamycin on the synthesis and export of fat body proteins and glycoproteins in larvae of the greater wax moth Galleria mellonella (L.). Insect Biochem. 12(3): 301-309. 1982.

Friedlander, A., Navarro, S. and Silhacek, D. L. The effect of carbon dioxide on NADPH production in Ephestia cautella (Walker). J. Comp. Biochem. Physiol. (Accepted October 1983)

Vick, K., Coffelt, J., Silhacek, D., and Oberlander, H. Methoprene and sex pheromone as control agents for the almond moth on stored in-shell peanuts. J. Econ. Entomol. (Accepted October 1984

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3. Number and title of CRIS work unit:

7712-20370-012 - Bioregulation of fetal, neonatal growth of pig adipose and skeletal muscle tissues.

4. Approach Element and Problem Definitions:

3.3.03.1.A

Objective	3	Productivity/Quality—Animals
Approach	3	Anim Nutrition/Feed Efficiency
Approach Element	03	Synth/Composition—Nutr/Physiol

Problem 1

The absence of defined methods for modifying the complex systems that synthesize the nutrients in animal tissues prevents the exercise of control over the proportions of those nutrients.

Subproblem a

Lack of knowledge of the metabolic systems, cellular systems, and mechanisms that control the rate and composition of protein and fat deposition in animals impedes progress to produce quality meat and fiber products of desirable composition at the lowest possible cost.

5. Estimated Sy's:

2.0

6. Objectives of research:

a. Determine the regulation of myonuclear proliferation in the fetal pig by locally produced growth factors and by blood-borne polypeptide inhibitory factors.

b. Identify factors that control proliferation and differentiation of adipocytes and stromal-vascular cells in fetal adipose tissue.

7. Research priorities in program:

a. Development of fractionation procedures to isolate at least semi-pure fractions of sera either rich in motogenic inhibitors and/or depleted of mitogenic stimulators of muscle and adipocyte proliferation.

b. Develop expertise in scanning and transmission electron microscopy of frozen tissue.

8. Progress of current research in solving problems:

Sera from fetuses of genetically lean and obese sows and sera from fetal and normal and hypophysectomized growing pigs were tested for mitogenic activity in cell culture using rat L-6 myoblasts. Replication of preadipocytes in response to sera from normal and hypophysectomized pigs was also examined. In terms of decreasing mitogenic potency, sera ranked normal > hypophysectomized > lean fetal = obese fetal for effect on myoblast proliferation. Preadipocyte proliferation was lower in presence of hypophysectomized pig serum when compared to normal pig serum. As a corollary, it was shown that enzyme histochemistry was useful for delineating adipocyte differentiation of stromal-vascular cells in primary culture. Studies have identified sera that are depleted in mitogenic factors and/or rich in mitogenic inhibitors.

9. Significant research accomplishments in the past 3 years:

As part of a basic research program to elucidate mechanisms of carcass fat deposition, the developmental biochemistry and anatomy of subcutaneous adipose tissue is being investigated. Several clear relationships between the structural aspects of adipose tissue (e.g., blood vessels, hair follicles) and adipocyte or fat cell development have been identified. For example, structural and histochemical changes in capillaries of the growing rat were associated with a decreasing rate of adipocyte hypertrophy. As blood-borne factors (e.g., hormones) become increasingly available through the elaboration and maturation of the capillary bed, the fat cells respond by decreasing the rate at which they fill with lipid. Thus, elaboration and inherent capabilities of blood vessels may be primary factors in causing obesity in meat animals and in man. These studies have application to the long-range goal of increasing efficiency of growth by decreasing carcass fat content.

As part of a basic research program to elucidate mechanisms that regulate carcass lean deposition, muscle metabolism and the proliferation of nuclei in the multinucleated myofiber are being investigated. The hypothesis that abnormalities in skeletal muscle metabolism could result in the onset of obesity was investigated in the pig. Lean and obese pigs were sampled at 110 days gestation when the body composition was similar. Although several histochemical and metabolic traits were influenced by phenotype, it remains to be established if these trait differences caused the later onset of obesity or if the results were related to endocrine imbalance or other factors. The skeletal muscles of obese mice weighed less than the muscles of lean mice. This was postulated to be the result of lower mitotic activity in the muscle satellite cells (nuclei of daughter

satellite cells are incorporated into muscle fibers as they grow in size). Factors that cause satellite cells to undergo mitosis must be identified. Studies to determine mechanisms that regulate muscle growth and mass have application to the long-range goal of increasing efficiency of growth by increasing carcass lean content.

To be capable of studying animals with the genetic potential for obesity in the preobese state is critical to the identification of factor(s) that trigger the onset of obesity. Fetuses from lean and obese pigs at 110 days of gestation present such a model because body composition is similar at this time. Yet, we have learned that the preobese animal can be identified well in advance of classical symptoms of obesity. Fat cell histochemistry for lipoprotein lipase completely distinguished between lean and obese fetuses despite similar cell sizes. Muscle composition also distinguished lean from obese fetuses; metabolic differences were also observed. However, rates of fatty acid oxidation and esterification were similar in muscle of lean and obese fetal pigs. But fatty acid oxidation/esterification ratio suggested that deposition of fatty acids as lipid was preferred in the muscle of obese neonatal pigs. This situation and the finding that rates of substrate oxidation and glycolytic flux were lower in muscle of obese pigs may be associated with onset of obesity in this polygenetic model. Muscle fiber diameters were similar fetally for lean and obese pigs. But muscles and fiber diameters were larger in the obese neonatal pig than in the age-matched lean pig.

10. Impact of research accomplishments on science and the general public:

There is a need as stated in Objective 3 of the ARS Program Plan to develop the means for increasing the productivity of animals and the quality of animal products. The specific strategic plan category is 3.3.03.1.A. While the objectives are basic in nature, achievement of these objectives will lay the foundation of knowledge upon which the producer will be able to increase meat animal productivity. Increased productivity could be realized through development/selection of animals with faster rates of gain, that are more efficient nutrient utilizers, and that have optimum lean to fat ratios. The research knowledge will also have application to human health problems, especially in the area of fetal and neonatal growth regulation.

11. Obstacles to achieving objectives:

To fully implement the program will require additional scientific support with accompanying technical support. Specifically, this would require two additional research physiologists to study (1) the physiology of growth and development of fat cells in culture, and (2) to investigate physiology of nutrient partitioning into lean and fat.

12. Future lines of needed research and plan for implementation:

The physiological and endocrinological mechanisms responsible for nutrient partitioning between lean and fat require additional research effort. Without additional support with ARS resources, a minimal research effort will be implemented through cooperative work with the Department of Foods and Human Nutrition at the University of Georgia.

13. Research facilities and personnel needs:

Existing facilities have the potential to accommodate an expanded program. Two additional SY's and four technical support personnel are required for a fully implemented program at the Richard B. Russell Agricultural Research Center Animal Physiology Research Unit.

14. Extent of cooperation--names of persons and institutions:

Dr. Roy Martin - University of Georgia
Dr. Robert Seerley - University of Georgia
Dr. William Vandergrift - University of Georgia
Dr. Roger Stone - Roman L. Hruska MARC, USDA-ARS, Clay Center, NE

15. Titles of publications for the last 3 years:

Campion, D. R., H. L. Marks, J. O. Reagan and J. B. Barrett. Composition and muscle cellularity of Japanese quail after selection for high body weight under optimal and suboptimal dietary conditions. Poultry Sci. 61:212-217. 1982.

Campion, D. R., H. L. Marks and R. L. Richardson. An analysis of satellite cell content in the semimembranosus muscle of Japanese quail (Coturnix coturnix japonica) selected for rapid growth. Acta Anat. 112:9-13. 1982.

Hughes, B. J., J. M. Bowen, D. R. Campion and W. E. Bradley. Influence of castration and denervation on bulbocavernosus muscle (BCM). Fed. Proc. 41:1595. 1982. (Abstract).

Jewell, D. E., D. R. Campion, D. Phillips and R. J. Martin. Changes in Zucker amino acid profiles due to different dietary protein concentrations. Fed. Proc. 41:712. 1982. (Abstract).

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3. Number and title of CRIS work unit:

Old CRIS - 7712-20370-002 - Endocrinological and Physiological Regulation of Reproductive Efficiency in Swine (in process of termination)

New CRIS - 6612-20370-015 - Bioregulation of the hypothalamo-hypophysial-gonadal axis in the pig (currently in approval process)

4. Approach Element and Problem Definitions:

3.2.01.1.A

Objective	3	Productivity/Quality--Animals
Approach	2	Effic-Reprodn & Relatd Processes
Approach Element	01	Offspring Reared Per M & F Maintnd

Problem 1
Reproductive inefficiency reflects the low number of offspring reared per female animal and is a major cause of low productivity in the livestock industry.

Subproblem a
Inability to detect and monitor the exact sequence of physiological events, from estrus to conception, limits efforts to increase the number of offspring per female per year.

5. Estimated SY's:

1.0

6. Objectives of research:

a. Determine the neuroendocrine mechanisms regulating luteinizing hormone and prolactin secretion in the pig.

b. Determine the peptide hormone requirements for follicular development.

c. Determine the complex of peptide hormones responsible for corpus luteum function during pregnancy.

7. Research priorities in program:

a. Develop expertise in stereotaxic placement of cannulae and electrodes in specific areas of the hypothalamus and limbic system of the central nervous system.

b. Develop radioimmunoassay and high pressure liquid chromotography procedures to quantitate neurotransmitters and endogenous opioid peptides in cerebral spinal fluid and neural tissue.

8. Progress of current research in solving problem:

The first investigation is now in progress to determine the effect of naloxone (NAL), an opiate antagonist, on luteinizing hormone (LH) and prolactin (PRL) secretion during the luteal phase of the estrous cycle. Preliminary results indicate, like all other species studied, NAL stimulated LH secretion, but unlike most other species studied, NAL also stimulated PRL secretion. A stereotaxic unit for the pig has been acquired and stainless steel cannulae are being fabricated.

9. Significant research accomplishments in the past 3 years:

Influence of Temperature on Blood Prolactin Concentrations in the Pig.

The postpartum interval of the sow is longer, and there is a higher incidence of anestrus and irregular estrous cycles in the summer than at other seasons of the year. There are also published reports that season of birth affected the age at puberty in gilts. Because hypersecretion of PRL has been demonstrated to suppress LH secretion as well as the ovarian response to gonadotropins in several species, and PRL secretion is highest in the pig and other species in the summer months, we have investigated the effect of photoperiod and temperature on PRL secretion in the pig. Our previous work demonstrated that photoperiod alone failed to alter serum concentrations of PRL, LH, GH and cortisol during lactation and PRL in the ovariectomized (OVX) gilt. Results of our recent work have demonstrated that temperature alone failed to alter serum PRL concentration in OVX gilts. We suggest that further work must be done to determine if photoperiod and temperature must be increased or decreased simultaneously to alter PRL secretion and, perhaps, reproduction efficiency in the pig.

Characterization of Normal Hormonal Events During Onset of Puberty in the Gilt.

The ovary responds to follicle stimulating hormone (FSH) and LH injections by 100 days of age, while the pituitary gland responds to injections of gonadotropin releasing (GnRH) and the hypothalamus (Brain)-pituitary axis responds to estrogen (E) as early as 60 days of age. Yet, these systems do not assume their normal temporal relationships to stimulate puberty until approximately 200 days of age. We determined serum LH and E concentrations during the weeks preceding normal onset of puberty through the use of frequent sequential blood sampling. Immediately preceding puberty, both LH and E secretion increased

significantly and LH secretion was characterized by increased frequency of lower amplitude LH release compared to previous ages. These results suggested that injections of small quantities of GnRH at intervals of one or two hours might stimulate a precocious onset of estrus and ovulation and overcome problems such as confinement-induced delayed puberty. Prepuberal gilts, 164 days of age, were injected (i.v.) hourly with 1 μg GnRH for 6 or 7 days. All 3 GnRH treated gilts exhibited estrus and ovulated within 6 days after treatment began while 0 of 3 control gilts remained prepuberal.

Temporal Relationships Between Serum PRL, LH and E During the Periestrous Period in Mature Gilts and in Prepuberal Gilts Induced to Ovulate.

We previously demonstrated that corpora lutea (CL) formed after induced ovulation with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) in prepuberal gilts are different from those formed during spontaneous ovulation in mature gilts. Induced CL are more sensitive to lysis either induced by uterine fragments after partial hysterectomy or exogenous $PGF_{2\alpha}$. Exogenous E or HCG caused luteal maintenance in mature gilts but caused luteal regression in prepuberal gilts. Therefore, the temporal relationships between serum PRL, LH and E concentrations during the periestrous period were compared between mature gilts and prepuberal gilts induced to ovulate. Examination of the data revealed that the periestrous serum PRL surges observed in the mature gilts were either totally absent or only partially present in the prepuberal gilts. These results indicate that abnormal CL function may result from inadequate exposure of pre-ovulatory follicles to PRL. Perhaps problems of early pregnancy failure in mature gilts is also caused by abnormal PRL secretion during the periestrous period.

10. Impact of research accomplishments on science and the general public:

The need for this research is documented in Objective 3 of the ARS Program Plan: to develop the means for increasing the productivity of animals through improved efficiency of reproduction and related processes. The specific strategic plan category is 3.2.01.1.A. Although the objectives of this CRIS project are basic in nature, achievement of these objectives will form the foundation upon which practical methods could be developed to increase reproductive efficiency in the pig. It has been previously demonstrated in the gilt that by 100 days of age the ovary responds to gonadotropin injections, while the pituitary responds to GnRH, and the hypothalamus(brain)-pituitary axis responds to E as early as 60 days of age. Yet, these systems do not assume their normal temporal relationships to cause puberty until approximately 200 days of age. The postpartum interval of the sow is longer, and there is a greater incidence of anestrus and irregular estrous cycles in the summer than at other seasons of the year. There are also published reports that season of birth affects age at puberty in gilts. In order to develop systems to accelerate onset of puberty and to overcome inefficiencies such as delayed puberty, irregular estrous cycles and prolonged postpartum intervals caused by environment, nutrition, disease or genetics, we must: (1) understand the endocrine events associated with onset of puberty, estrus, ovulation, CL maintenance and termination of the postpartum interval, and (2) determine the bioregulatory mechanisms controlling the brain-pituitary-gonadal axis.

11. Obstacles to achieving objectives:

To fully implement the program will require additional scientific support with accompanying technical support. Specifically, this would require two research physiologists to study physiology of reproduction in the female pig under various managment systems. Bioregulatory mechanisms of the neuroendocrine system which control onset of puberty, length of postpartum anestrus, the estrous cycle and ovulation would be studied.

12. Future lines of needed research and plan for implementation:

Nutritional effects on pituitary-ovarian function has been neglected. At this time work has been initiated at Mississippi State University, North Carolina State University and University of Georgia through cooperative agreements using funds from the ARS Integrated Reproduction Management program.

13. Research facilities and personnel needs:

Existing facilities have the potential to accommodate an expanded program. Two additional SY's and four technical support personnel are required for a fully implemented program at the Richard B. Russell Agricultural Research Center Animal Physiology Research Unit.

14. Extent of cooperation--names of persons and institutions:

Dr. George Rampacek - University of Georgia
Dr. Terry Kiser - University of Georgia
Dr. Fred Thompson - University of Georgia
Dr. Dennis Marple - Auburn University
Dr. Nancy Cox - Mississippi State University
Dr. Rick Jones - University of Georgia
Dr. Jack Britt - North Carolina State University
Dr. Robert Collier - University of Florida
Dr. Fuller Bazer - University of Florida

15. Titles of publications for the last 3 years:

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Fonda, E. S., G. B. Rampacek and R. R. Kraeling. The effect of adrenocorticotropin or hydrocortisone on serum luteinizing hormone concentrations following adrenalectomy and/or ovariectomy in the prepuberal gilt. Endocrinol. 114:268-273. 1984.

Lutz, J. B., G. B. Rampacek, R. R. Kraeling and C. A. Pinkert. Serum luteinizing hormone and estrogen profiles prior to puberty in the gilt. J.Anim. Sci. 58:686-691. 1984.

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Kraeling, R. R., C. R. Barb and G. B. Rampacek. Ovarian response of the hypophysial stalk transectomized pig to pregnant mare serum gonadotropin. (In editorial review).

Kraeling, R. R., G. B. Rampacek and C. R. Barb. Failure of the anterior pituitary to respond to gonadotropin releasing hormone after hypophysial stalk transection in the gilt. (In editorial review).

Kraeling, R. R., G. B. Rampacek and C. R. Barb. Luteinizing hormone secretion after hypophysial stalk transection and exogenous estrogen in the ovariectomized gilt. (In editorial review).

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2. **Location:**

New Orleans, Louisiana

3. **Number and title of CRIS work unit:**

6435-20560-005 - Biologically Active Peptides Controlling Functional Determinants of Plant/Animal Quality Parameters

4. **Approach Element and Problem Definitions:**

4.1.02.1.1C

5. **Estimated SY's:**

Four

6. **Objectives of research:**

Characterize the mechanisms involved in biosynthesis of microbial peptides through cloning of pertinent genes and expression in appropriate bacterial species. Identify the conditions for optimum production of specific peptides in axenic fermentation to provide quantities of material required for comprehensive chemical and biological characterization. Elucidate processes associated with accumulation of biologically active peptides and elicited allelochemicals in edible portion of crop plant tissues and assess the implications of their presence in food/feed in the context of pest control and safety.

7. Research priorities in your program:

1. To isolate and characterize bioactive peptides present in fungal and plant systems.

2. To elucidate the role of bioactive peptides in plant regulatory control mechanisms.

3. To investigate structure-actively relationships in peptide receptor interactions with a view to both elucidating pertinent plant biochemical mechanism and developing economical herbicides, insecticides, and fungicides.

8. Progress of current research in solving problems:

We have only begun equipping our laboratories and are involved presently in developing procedures for scaling-up the fermentation production of tentoxin, a cyclic tetrapeptide. We are also initially involved in the chemical synthesis of tentoxin and its chemical intermediates.

9. Significant research accomplishments in the past 3 years:

The project is just in its beginning stages.

10. Impact of research accomplishments on science and the general public:

The project is just in its beginning stages.

11. Obstacles to achieving objectives:

The project is just in its beginning stages.

12. Future lines of needed research and plan for implementation:

The project is just in its beginning stages.

13. Research facilities and personnel needs:

The project involves four SYs and six support positions scheduled for FY 85.

Future major item acquisitions would include a solid phase peptide synthesizer which would accelerate peptide research in ARS through expediting synthesis of peptides needed in the study of insect and plant systems.

14. Extent of cooperation--names of persons and institutions:

Discussion of future collaboration with Dr. Howard Jaffe, Dr. Edward P. Masler, Dr. Benjamin Cook, Dr. Dora Hayes and Dr. Al DeMilo was initiated.

15. Titles of publications for the last 3 years:

The project is just in its beginning stages.

1. Scientist's name, address, and telephone number:

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2. Location:

Helminthic Diseases Lab.
Animal Parasitology Inst.
BARC-East

3. Number and title of CRIS work unit:
1205-20493-009 Characterization of unique epitopes of membrane proteins that regulate host/parasite interactions.

4. Approach Element and Problem Definitions:

Analyze immune response gene control of antibody production and immune cellular interactions after stimulation with proteins or their peptides; looking for peptide determinants that stimulate helper or suppressor immune pathways.

5. Estimated SY's:

1.0 (+1.0 postdoctoral fellow - requested 10/84)

5a. Technical capabilities + Equipment:

Facilities for growth, production, and characterization of monoclonal antibodies.

6. Objectives of research:

(1) Analyze immune responses of swine to parasitic infection (both cellular interactions and immunogenetic control).

(2) Analyze genetics of swine major histocompatibility complex.

(3) Probe immune response gene control of responses to protein/peptide immunization or parasite infection.

(4) Identify immunodominant epitopes of parasite or membrane proteins.

(5) Probe helper/suppressor cell pathways following immune stimulation.

7. Research priorities in your program:

Analyze immune responses in swine at cellular level by analysis of cell surface molecules, and at genetic level by analysis of antibody and cellular responses.

8. Progress of current research in solving problems:

(1) Preliminary studies of cell subpopulations involved in responses to Ascaris suum infection indicate that macrophages and B cells show significant variations with immune status to this parasite infection.

(2) New monoclonal antibodies to swine MHC atigents are being analyzed for their biochemical and immunological importance.

(3) Antibodies are being developed to screen embryos prior to introducing new genetic material.

(4) Antisera to protein antigens are being screened for reactivity to specific peptides.

9. Significant research accomplishments in the past 3 years:

(1) Production of part of monoclonal antibodies to swine peripheral blood lymphocyte subsets.

(2) Identification of immune response gene control of antibody and cellular responses to protein antigens.

(3) Purification and characterization of swine major histocompatibility antigens for sequence analysis and x-ray criptallography studies.

10. Impact of research accomplishments on science and the general public:

Biotechnologically engineered vaccines usually consist of a single peptide or protein. These vaccines must be analyzed for genetic control of antibody production and of cellular stimulation. Certain peptides could cause stimulation of only the suppression or helper pathways and thus cause under-or reproduction of specific immune factors.

11. Obstacles to achieving objectives:

Too many projects - too few people - not enough time on cell sorter.

12. Future lines of needed research and plan for implementation:

Study of suppression cell pathways - analyze immune response to protein/peptides or to parasites see if suppressor cells are stimulated and analyze how they can be controlled.

13. Research facilities and personnel needs:

Postdoctoral fellow - to begin work on analysis of epitopes of proteins that stimulate helper or suppressor immune pathways.

FACS analyzer (flow microfluorimeter) - to analyze cell markers and % of cells produced by different immunization/infection/vaccination protocols.

14. Extent of cooperation--names of persons and institutions:

NIH (1) Drs. Mark Pescovitz and David Sachs, Immunology Branch NCI: MAb production and characterization.

(2) Drs. David Davies and Eduardo Padlan, NIAMDD--crystallization of SLA molecules.

(3) Dr. Dinah Singer.

USDA (1) Drs. Vern Pursel, Bob Wall and Caird Rexroad--Introduction of swine SLA genes to embryos.

(2) Mr. Julio Quintero, PIADC--uptake of pig cell markers by RBC infected with ASFV.

Wash.State, Pullman, WA
(1) Dr. William Davis--analysis of MAb speacific for pig PBL.

15. Titles of publications for the last 3 years:

Metzer, J.-J., Lunney, J. K., Sachs, D. H. and Rudikoff, S. Transplantation in miniature swine. XII. N-terminal sequences of Class I histocompatibility antigens (SLA) and beta$_2$-microglobulin. J. Immunol. 129:716-721, 1982.

Thistlethwaite, J. R., Pennington, L. R., Lunney, J. K., and Sachs, D. H. Transplantation in miniature swine. XIII. Immunological characterization of two recombinanat SLA haplotypes. Transplantation 35:394-400, 1983.

Lunney, J. K., Osborne, B. A., Devaux, C., Pierres, M., and Sachs, D. H. Interspecies cross-reactivity of monoclonal anti-I-E antibodies specific for polymorphic Ia determinants. In Pierce, C., et al. (Eds.) Ir Gene: Past, Present and Future, Clifton, NJ, The Humana Press, Inc., 1983, pp. 57-61.

Lunney, J. K., Osborne, B. A., Devaux, C., Pierres, M., and Sachs, D. H. Interspecies cross-reactivity of monoclonal antibodies directed against polymorphic mouse Ia determinants. Transplant. Proc. 15:139-141, 1983.

Thistlethwaite, J. R., Jr., Auchincloss, H., Jr., Lunney, J. K., Pennington, L. R., Pescovitz, M. D., and Sachs, D. H. Transplantation in miniature swine. In vitro testing and renal allograft survival in SLA-D matched swine. Transplant. Proc. 15:152-155, 1983.

Lunney, J. K., Osborne, B. A., Sharrow, S. O., Devaux, C., Pierres, M., and Sachs, D. H. Sharing of Ia antigens between species. IV. Interspecies cross reactivity of monoclonal antibodies directed against polymorphic mouse Ia determinants. J. Immunol. 130:2786-2793, 1983.

Osborne, B. A., Lunney, J.K., Pennington, L. R., Sachs, D. H., and Rudikoff, S. Two dimensional gel analysis of gene products of the miniature swine major histocompatibility complex. J. Immunol., 131:2939-2944, 1983.

Pescovitz, M. D., Sachs, D. H., Lunney, J. K., and Hsu, S. M. Localization of class II MHC antigens on porcine renal vasular endothelium. Transplantation 37:627-631, 1984.

Pescovitz, M. D., Lunney, J. K., and Sachs, D. H. Preparation and characterization of monoclonal antibodies reactive with porcine PBL. J. Immunol. 133:368-375, 1984.

Papers in Press 1984

Lunney, J. K. Use of monclonal antibodies for the analysis of immune responses in swine. In Stern, N. J. and Gamble, H. R. (eds.) Hybridoma Technology in Agricultural and Veterinary Research. Rowman and Allenheld, Totowa, NJ. pp 298-301

Pescovitz, M. D., Lunney, J. K., and Sachs, D. H. Murine anti-swine T4 and T8 monoclonal antibodies: Distribution and effects on proliferation and Cytotoxic T cells. J. Immunol.

Papers Submitted 1984

Lunney, J. K., Metzger, J.-J., Rudikoff, S., and Sachs, D. H. Transplantation in miniature swine. XI. Purification of Nonidet P40 solubilized class I histocompatibility antigens.

Epstein, S. E., and Lunney, J. K. A cell surface ELISA assay in the mouse using only poly-L-lysine as a fixative. J. Immunol. Meth. (in press).

Papers in Preparation

VanderPutten, D., Pescovitz, M. D., and Lunney, J.K. Imune response gene control of antibody and cellular responses to (T-G)-A--L and lysozyme in the swine.

Lunney, J. K., and Osborne, B. A. Cell surface ELISA assay for Ia activity.

Pescovitz, M. D., Lunney, J. K., and Sachs, D. H. Differential expression of Ia antigens on porcine helper and cytotoxic T cell subsets.

JOAN K. LUNNEY

Current Research

1. Use of monoclonal antibodies to lymphocyte subpopulations to characterize the immune response of swine immunized against the parasite, Ascaris suum. Analyze the responding cell populations a) as a measure of predicting the status of protection against subsequent infections or b) as a means of modulating the responding cell populations to alter the immune response.

2. Use of miniature swine that are inbred at their major histocompatibility complex (MHC) to analyze the effects of these genes on the course of trichinella infections (study larval recovery, adult fecundity, host blastogenic response and antibody titer). Future use of these swine to analyze the effects of MHC genes on vaccine responses.

3. Biochemical and immunologic characterization of MHC antigens in miniature swine. Production and/or characterization of monoclonal antibodies reactive with subsets of these antigens. Large scale purification and biochemical analyses of these cell surface proteins.

1. Scientist's name, address, and telephone number:

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Insect Reproduction Laboratory
BARC-East 306, Beltsville, MD 20705
344-2136 (FTS)

2. Location:

Insect Reproduction Laboratory
Beltsville, MD

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction

2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

6.7

6. Objectives of research:

Isolate and identify insect neuroregulators and determine their mode of action. Utilize this knowledge for developing new procedures for controlling insects.

7. Research priorities in your program:

Isolate and identify the mosquito egg development neurosecretory hormone. Determine its relationship with other neurohormones and regulators. Develop bioassays suitable for isolating neuroregulators of spermatogenesis.

8. Progress of current research in solving problems:

Antibodies against a 500-fold purified EDNH fraction are being prepared in newly established immunological facility in this laboratory. Histochemical evidence was obtained for neurosecretion that coincides with changes in testicular activity in synthesizing ecdysteroids. This activity, which can also be regulated by brain extracts or by appropriately timed decapitation, may be utilized as a bioassay for the isolation of spermatogenesis-regulating neurohormones.

9. Significant research accomplishments in the past 3 years:

Established a comprehensive research program for the isolation and characterization of neuroregulators involved in insect reproduction and for the elucidation of their mode of action. A bioassay for one of them, the mosquito EDNH, has been perfected and utilized for 500-fold purification of the neuropeptide. During this process, a new EDNH-inhibiting factor was discovered. Initiated a research program for elucidating the regulation of spermatogenesis in Lepidoptera. A significant accomplishment in this project was the discovery that testes synthesize ecdysteroids and that this process can be utilized for developing bioassays for neuroregulators of spermatogenesis. The research project on the elucidation of neuroregulation of oogenesis led to the discovery of a new molting hormone for hemipterous insects. Directed a research program in immunology that resulted in the first preparation of monoclonal antibodies against mosquito vitellogenins.

10. Impact of research accomplishments on science and the general public:

The accomplishments highlighted in the previous section had impact primarily on insect neuroscience by confirming the role of the nervous system as a biochemical regulator of reproduction. Especially in the field of spermatogenesis, first evidence was developed implicating the brain's regulatory activity. In addition, the accomplishments indicated new possibilities and directions for exploiting neuroregulation and its disruption for insect control purposes. This aspect of the research was emphasized in several recent press articles.

11. Obstacles to achieving objectives:

To achieve objectives, current research will require a stronger financial base. Despite a budget increase in FY85, research funds (All Other category) decreased by $7,000 compared with FY84.

12. Future lines of needed research and plan for implementation:

The neuropeptide EDNH will be purified and identified with the aid of antibodies that are now being developed. The availability of pure EDNH will allow further work on crossreactivity with PTTH, characterization of the EDNH-inhibiting factor, and a confirmation of possible other forms of EDNH in Diptera and other insects. Continued development of bioassays for the spermatogenesis-regulating brain factors will make possible their isolation and identification. The development of new projects on genetic engineering of insect brain hormones is needed. These projects will be initiated and implemented if resources for such work become available.

13. **Research facilities and personnel needs:**

The laboratory needs one SY (Res. Biochemist) to acquire expertise and capability to pursue work on neuropeptides in depth. Initial plans for including two SY-biochemists to the staff were not implemented. There is urgent need for providing two postdoctoral positions for research on neuropeptides. One such position is currently being requested (ARS Postdoctoral Research Associate).

14. **Extent of cooperation--names of persons and institutions:**

The laboratory cooperates extensively with ARS and other scientists. Partial listing follows:

R. H. Lawson, FNCL/HSI, BARC
C. H. Lorreau, NCL/HSI, BARC
T. Adams, MRRL, Fargo, ND
E. Schafer, F&WLS, USDI, Denver, CO
H. D. Guthrie, RL/ASI, BARC
D. K. Hayes, LIL/AEQI, BARC
R. H. Miller, MSML/ASI, BARC
R. Wagner, VTERL, College Station, TX
W. E. Bollenbacher, U. North Carolina, Chapel Hill, NC
M. Ma, U. Maryland, College Park, MD
K. Baldwin, Howard Univ., Washington, DC
R. B. Imberski, U. Maryland, College Park, MD
S. C. Saxena, U. Rajasthan, Jaipur, India
E. C. Rubenstein, Skidmore College, Saratoga Spring, NY
S. W. Appelbaum, Hebrew Univ., Rehovot, Israel

15. **Titles of publications for the last 3 years:**

In 1982-1984, IRL scientists (6.7 SY) published 57 papers and one book. My personal publications are on the enclosed sheet.

162. Borkovec, A. B. 1982. In praise of Viola corsica. Bull. Amer. Rock Garden Soc. 40: 26.

163. Kelly, T. J., R. E. Redfern, A. B. DeMilo, and A. B. Borkovec. 1982. Inhibition of ecdysis in Oncopeltus fasciatus by 2-acetylpyridine thiosemicarbazones. Pestic. Biochem. Physiol. 17: 35-41.

164. Borkovec, A. B. 1982. Insect chemosterilants: Retrospects and prospects. Amer. Chem. Soc. 16th Great Lakes Regional Mtg., Abstracts, p. 49.

165. Borkovec, A. B., and C. W. Woods. 1982. Alkylating agents. In Handbook of Carcinogens and Hazardous Substances, M. C. Bowman (ed.), Marcel Dekker, New York, 750 pp., 19-74.

166. Loeb, M. J., C. W. Woods, E. P. Brandt, and A. B. Borkovec. 1982. Larval testes of the tobacco budworm: a new source of insect ecdysteroids. Science 218: 896-8.

167. Kelly, T. J., C. W. Woods, M. J. Birnbaum, and A. B. Borkovec. 1982. Physiological function and partial purification of an ovarian maturation inhibitor from the housefly, Musca domestica. Joint Mtg. Entomol. Soc. Amer., Can. Ont., Toronto, Abstract 649.

168. Redfern, R. E., T. J. Kelly, A. B. Borkovec, and D. K. Hayes. 1982. Ecdysteroid titers and molting aberrations in last-stage Oncopeltus nymphs treated with insect growth regulators. Pestic. Biochem. Physiol. 18: 351-6.

169. Borkovec, A. B. 1983. Chemicals for control of insect reproduction. Beltsville Symp. VIII, Agric. Chem. Future, Abstract 54.

170. Pessah, I. N., R. E. Menzer, and A. B. Borkovec. 1983. Activity and fate of 2,4-diamino-6-(2-furyl)-s-triazine in female house flies: Indication of a novel mode of action. Beltsville Symp. VIII, Agric. Chem. Future, Abstract 21.

171. DeMilo, A. B., R. E. Redfern, and A. B. Borkovec. 1983. 2-Acetylpyridine thiosemicarbazones as inhibitors of ecdysis in Oncopeltus fasciatus: Structure-activity relationship study. J. Agric. Food Chem. 31: 713-8.

172. Kelly, T. J., C. W. Woods, M. J. Birnbaum, and A. B. Borkovec. 1983. Oostatic hormone and biogenic amines as inhibitors of ovarian maturation in house flies and mosquitoes. Internat. Conf. Insect Neurochem. Neurophysiol., College Park, MD. Abstract 27.

173. Masler, E. P., H. H. Hagedorn, D. Petzel, and A. B. Borkovec. 1983. Preparation of egg development neurosecretory hormone using reverse phase chromatographic techniques. Internat. Conf. Insect Neurochem. Neurophysiol., College Park, MD. Abstract 29.

174. Borkovec, A. B. 1983. Insect chemosterilants derived from plants. Internat. Conf. Nat. Products as Regulators of Insect Reprod., Jammu, India, Abstracts.

175. Masler, E. P., H. H. Hagedorn, D. H. Petzel, and A. B. Borkovec. 1983. Partial purification of egg development neurosecretory hormone with reverse-phase liquid chromatographic techniques. Life Sci. 33: 1925-31.

176. Borkovec, A. B. 1983. In praise of Hymenoxys scaposa. Bull. Amer. Rock Garden Soc. 41: 195-6.

177. Kelly, T. J., C. W. Woods, M. J. Birnbaum, and A. B. Borkovec. 1984. Oostatic hormone and biogenic amines as inhibitors of ovarian maturation in house flies and mosquitoes. In Insect Neurochemistry and Neurophsiology, A. B. Borkovec and T. J. Kelly (eds.), Plenum Publ. Corp., New York, 401-3.

178. Masler, E. P., H. H. Hagedorn, D. H. Petzel, and A. B. Borkovec. 1984. Preparation of egg development neurosecretory hormone using reverse-phase liquid chromatographic techniques. In Insect Neurochemistry and Neurophysiology, A. B. Borkovec and T. J. Kelly (eds.), Plenum Publ. Corp., New York, 427-30.

179. Borkovec, A. B., and T. J. Kelly (eds.) 1984. Insect Neurochemistry and Neurophysiology. Plenum Press, New York, London, 523 pp.

180. Kelly, T. J., M. J. Birnbaum, C. W. Woods, and A. B. Borkovec. 1984. Effects of house fly oostatic hormone on egg development neurosecretory hormone action in Aedes atropalpus. J. Exp. Zool. 229: 491-6.

1. Scientist's name, address, and telephone number:

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(301) 344-2923

2. Location:

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Beltsville, Maryland

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

1.0

6. Objectives of research:

To develop novel, easily synthesizable, chemical agents that interfere with the insect reproduction processes of insects or with their normal growth and development. Optimally these agents will possess low mammalian toxicity, high species specificity, and negligible environmental impact as a requirement for their utilization in new pest control strategies.

6a. Technical expertise/equipment:

Organic chemist familiar with a broad scope of organic reactions but most familiar with heterocyclic chemistry. Familiar with modern analytical and spectroscopic techniques required for isolation, purification and identification of organic compounds. Equipment: Hewlett-Packard 5995 GC/MS with D.I.P. Perkin-Elmer 299 grating infrared spectrophotometer. Perkin-Elmer 599 UV-VIS spectrophotometer. Waters 500 prep LC system.

7. Research priorities in your program:

Current focus on the development of model compounds capable of modulating neuropeptide synthesis, storage, release, transport and/or receptor binding. In cooperation with physiologists, is determining the mode of action of model compounds that induce physiological/morphological responses that suggest neuropeptide interference or regulation. Through chemical synthesis and structure-activity relationship studies is optimizing activity of "bioactives" and utilizes this information for the development of new technologies for pest control.

8. Progress of current research in solving problems:

1. Working in conjunction with physiologists to modify/streamline bioassays in O. fasciatus, P. interpunctella and M. domestica for their utilization in developing novel hormone agonists/antagonists to control reproduction.

2. Presently conducting structure-activity relationship studies on two new classes of developmental inhibitors; thiazolyureas and azocarboxamides.

3. Presently determining ecdysteroid and JH esterase profiles in H. virescens affected by growth modifier AI3-64341 (thiazolylurea).

9. Significant research accomplishments in the past 3 years:

1. Discovered a new class of insect molt inhibitors derived from 2-acetylpyridine thiosemicarbazone.

2. Discovered a class of nonterpenoid compounds (thiolcarbamates) that elicit the classical JH morphological effects in insects (mode of action unknown).

3. Discovered a new class of compounds (thiazolylureas) with insect growth regulating activity in moths (mode of action unknown).

10. Impact of research accomplishments on science and the general public:

Developments in item 9 are too new to accurately assess their impact but several companies have shown interest in these developments and have done some follow-up work on these materials.

11. Obstacles to achieving objectives:

1. Lack of specific and simple bioassays to identify compounds that directly interfere with neuropeptides or their possible cofactors.
2. Lack of technical personnel (none with me at present)
3. Increasing amount of administrative paper work is a major deterrent to increased productivity.

12. Future lines of needed research and plan for implementation:

As model compounds (small easily synthesized molecules) are specifically implicated in regulation of neuropeptide synthesis or functions the following long range lines of research (from an organic chemist's perspective) should be initiated: (a) conduct sophisticated quantitative structure-activity relationships studies to optimize activity; (b) conduct pharmacokinetic studies of radiolabelled compounds to elucidate the mode of action at the cellular and molecular level; (c) conduct studies to identify receptor molecules and critical binding sites. These studies will be implemented as sufficiently active neuropeptide inhibitors are identified.

13. Research facilities and personnel needs:

1. Urgently need one or more technical assistants
2. Need a microcomputer to assist in searching a large data base of previously tested compounds. Also, need it to search outside data bases of existing chemical literature.
3. Need an up-date in gas chromatographic equipment to allow for capillary column experiments useful for identifying extremely small quantities (subnanogram range) of natural products by GC/MS techniques.

14. Extent of cooperation--names of persons and institutions:

S. B. Haught, Insect Reproduction Laboratory
M. Loeb, Insect Reproduction Laboratory
T. J. Kelly, Insect Reproduction Laboratory
E. P. Masler, Insect Reproduction Laboratory
D. Gelman, Insect Reproduction Laboratory
R. E. Redfern, Livestock Insects Laboratory
M. M. Crystal, Livestock Insects Laboratory
R. Miller, Livestock Insects Laboratory
Hugh Sisler, Dept. of Botany, Univ. of MD, College Park
E. Schafer, Jr., Dept. of Interior, Fish and Wildlife Service, Denver, CO

15. Titles of publications for the last 3 years:

Kelly, T. J., R. E. Redfern, A. B. DeMilo and A. B. Borkovec. 1982. Inhibition of ecdysis in Oncopeltus fasciatus by 2-acetylpyridine thiosemicarbazones. Pestic. Biochem. Physiol. 17:35-41.

DeMilo, A. B. and R. E. Redfern. 1982. Further development in nonterpenoid insect juvenile hormone mimics derived from 4-(benzyloxy)benzene. 17th ACS MARM. Abstract No. 12, p. 15.

DeMilo, A. B., S. B. Haught, and T. J. Kelly. 1983. Non-terpenoid thiocarbamates with juvenoid activity. Beltsville Symp. VIII., Agricultural Chemicals of the Future, Abstract No. 5.

DeMilo, A. B., R. E. Redfern, and A. B. Borkovec. 1983. 2-Acetylpyridine thiosemicarbazones as inhibitors of ecdysis in Oncopeltus fasciatus: structure-activity relationship study. J. Agric. Food Chem. 31(4):713-718.

DeMilo, A. B., M. J. Loeb, and R. E. Redfern. 1983. Growth regulating effects of a thiazolylurea in two lepidoptera. 186th ACS Natl. Meeting, Pest. Div., Abstract No. 45.

M. M. Crystal and A. B. DeMilo. 1984. A laboratory method for evaluating acaricides against the northern fowl mite. J. Georgia Entomol. Soc., in press.

R. E. Redfern, D. K. Hayes, J. D. Warthen, A. B. DeMilo, and T. P. McGovern. 1984. Responses of nymphs of the large milkweed bug and pupae of the yellow mealworm to three compounds affecting insect growth. Proceed. 1st Intl. Montreaux Conf. of Biol. Rhythms. in press.

A. B. DeMilo. 1984. Nonterpenoid insect juvenile hormone mimics derived from 4-(benzyloxy)benzene. XVII Intl. Congr. of Entomol. Abstract, p. 860.

A. B. DeMilo, S. B. Haught, and T. J. Kelly. 1984. Nonterpenoid s-benzyl thiocarbamates with juvenile hormone-like activity: structure-activity relationships. 188th ACS Natl. Meeting, Pest. Div., Abstract No. 32.

1. Scientist's name, address, and telephone number:

Dale B. Gelman
Bldg. 306, Rm. 119
BARC-East
Beltsville, Maryland 20705

2. Location:

USDA,ARS,AEQI
Insect Reproduction Laboratory
Beltsville, Maryland 20705

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

.9

6. Technical Capabilities and Equipment

Radioimmunoassays for quantitating ecdysteroids, cyclic AMP, cyclic GMP, etc.
Sterile facilities for tissue and organ culture
Bioassay for the measurement of chitin synthesis

7. Objectives of research:

Develop bioassays for and characterize factors that regulate spermatogenesis especially as it is related to larval diapause. Determine the regulatory mechanisms involved. Assess the activity of compounds that might interfere with the regulation of spermatogenesis.

8. Research priorities in your program:

Identify and investigate the control mechanisms involved in shutting down spermatogenesis in diapause-bound animals and in reinitiating sperm development under diapause-break conditions.

9. Progress of current research in solving problems:

The time table of events involved in spermatogenesis under non-diapause-inducing, diapause-inducing and diapause-break conditions has been determined. Fluctuations of total ecdysteroid and individual ecdysteroids also have been determined and have been related to the progress of spermatogenesis. Times of production of head factors which influence the progress of both eupyrene and apyrene spermatogenesis have been identified.

10. Significant research accomplishments in the past 3 years:

(1) Developed a method for staging larvae and pupae of the European corn borer (ECB) so that observed anatomical and biochemical changes could be associated with a given physiological stage of development.

(2) Identified critical periods for PTTH and ecdysone release in last instar ECBs and uncovered a photoperiodic link to release of these hormones.

(3) Determined fluctuations of individual ecdysteroids in insect hemolymph and testes and related them to physiological events in insect development and metamorphosis.

(4) Developed a rapid and reliable assay for measuring chitin synthesis in the ECB male clasper.

11. Impact of research accomplishments on science and the general public:

Findings have provided valuable new information for the scientific community, served to elucidate the role of hormones and neurohormones in insect development and metamorphosis, established new concepts about the physiology and control of diapause in insects, and have opened up new avenues of investigation for future research in insect development, metamorphosis and reproduction. For the long term, findings can be used to develop novel, new biorational compounds for insect control and thus help to reduce the multibillion dollar losses due to the activity of insect pests.

12. Obstacles to achieving objectives:

Major obstacles are the same as those facing most members of the scientific community:

(1) valuable research time lost in handling administrative duties and paperwork
(2) insufficient technical help
(3) travel ceilings that interfere with cooperative efforts and receiving first-hand information at scientific meetings

13. Future lines of needed research and plan for implementation:

It is difficult to plan too far in the future since current and short term results must dictate future plans. However, depending upon our success in uncovering regulatory factors and characterizing these moieties, we would want to isolate and purify these regulators, develop rapid biochemical assays for their detection, determine their mechanism of action and develop insect-specific methods to interfere with this action.

14. Research facilities and personnel needs:

a) technical support help
b) conveniently located quiet area for writing and literary research activities

15. Extent of cooperation--names of persons and institutions:

1. Dr. Raziel Hakim
   Howard University
   Washington, DC

2. Dr. Hiram Larew, ARS
   Bldg. 470, Rm. 000
   Beltsville, MD

3. Dr. Marcia Loeb
   Insect Reproduction Laboratory
   Bldg. 306, Rm. 119
   Beltsville, MD

4. Dr. Charles Woods
   Insect Reproduction Laboratory
   Bldg. 306, Rm. 113
   Beltsville, MD

16. Titles of publications for the last 3 years:

Gelman, D.B. and D.K. Hayes. Methods and markers for synchronizing the maturation of fifth stage diapause-bound and nondiapause-bound larvae, pupae and pharate adults in the European corn borer, Ostrinia nubilalis. Ann. Entomol. Soc. Amer. 75:485-493. 1982.

Gelman, D.B. and D.K. Hayes. Critical periods for the brain and prothoracic glands of 5th instars of the European corn borer, Ostrinia nubilalis (Hubner). Comp. Biochem. Physiol. 73:81-87. 1982.

Gelman, D.B., C.W. Woods, and L. Brents. Hemolymph ecdysteroid titers: non-diapause vs diapause-bound fourth and fifth instars of the European corn borer, Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae). 54th Ann. Mtg. Eastern Branch Entomol. Soc. Amer., Hartford, Conn., September 1982, p. 40.

Gelman, D.B. and C.W. Woods. Haemolymph ecdysteroid titers of diapause- and nondiapause-bound fifth instars and pupae of the European corn borer, Ostrinia nubilalis (Hubner). Comp. Biochem. Physiol. 76:367-375. 1983.

Gelman, D.B. The influence of temperature on photoperiod: control of diapause induction in different strains of the European corn borer. International Conference on Neurochemistry and Neurophysiology, Abstract No. 48. 1983.

Gelman, D.B. and C.W. Woods. Distribution of ecdysteroids in hemolymph and testes of last instars, pupae and pharate adults of the European corn borer, Ostrinia nubilalis. American Zoologist 23:991. 1983.

Gelman, D.B. The influence of temperature on photoperiod: control of diapause induction in different strains of the European corn borer. In Insect Neurochemistry and Neurophysiology (A.B. Borkovec and T.J. Kelly, eds.). Plenum Press, N.Y. pp. 357-359. 1984.

Gelman, D.B. and L.A. Brents. Haemolymph ecdysteroid levels in diapause- and nondiapause-bound fourth and fifth instars and in pupae of the European corn borer, Ostrinia nubilalis (Hubner). Comp. Biochem. Physiol. 78:319-325. 1984.

1. Scientist's name, address, and telephone number:

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2. Location:

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Beltsville, MD 20705

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

0.6

6. Objectives of research:

1. Neuroendocrine regulation of spermatogenesis in Heliothis virescens
2. Functional morphology of neurosecretory cells in insects
3. Sex-specific development of the nervous system in Manduca sexta

7. Research priorities in your program:

All of the above

8. Progress of current research in solving problems:

1. Tissue culture studies are in progress to determine regulation of spermatogenesis by neurohormonal factors in controlled environments.
2. Development of immunocytochemical technique is in progress.
3. Several experiments are conducted to determine factors controlling sex-specific degeneration of neurons in Manduca

9. Significant research accomplishments in the past 3 years:

1. Correlation of ecdysteroid titers with 2 types of larval behavior in Ephestia kuchniella
2. Finding of sex-specific neuronal death during metamorphosis in Manduca sexta
3. Identification of neurosecretory cell innervating ring gland of the flesh fly larvae by cobalt back-filling techniqae.

10. Impact of research accomplishments on science and the general public:

Mechanisms of neuron's degeneration are of great interest since several human diseases are caused by degenerative changes in the nervous system. Insects with their big, identifiable neurons are very suitable model system in which to study control of neurons degeneration.

11. Obstacles to achieving objectives:

a) Lability of neural factors makes them difficult to handle and store.

b) Difficulty in rearing experimental animals in uniform quantities throughout the year makes performance of experiments ad libitum difficult.

12. Future lines of needed research and plan for implementation:

In future, neural factors should be isolated and characterized. Sufficient volumes of relatively pure factors should be readied for antibody production. Once antibodies are in hand, cytoimmunology to detect anatomical sites of synthesis and sites of receptors can be attempted. Cellular mechanisms of action can be studied.

13. Research facilities and personnel needs:

Research facilities: a) office and more lab space; b) computerized device for counting spermatocysts in tissue culture

Personnel: a) technician
b) student

14. Extent of cooperation--names of persons and institutions:

Staff of IRL
James Truman, Dept. of Zoology, Univ. of Washington, Seattle, WA

15. Titles of publications for the last 3 years:

Giebultowicz, J. M. and Saunders, D. S. 1983. Evidence for the neurohormonal basis of commitment to pupal diapause in larvae of Sarcophaga argyrostoma. Experientia 39:194-196.

Giebultowicz, J. M. and Truman, J. W. 1984. Sexual differentiation in the terminal ganglion of the moth Manduca sexta: role of sex specific neuronal death. J. Comp. Neurol. 226:87-95.

Giebultowicz, J. M., Cymborowski, B. and Delbecque, J. P. 1984. Environmental control of larval behavior and its consequences for the ecdysteroid content and pupation in Ephestia kuehniella. Physiol. Entomol., 9 (in press)

Stringer, I., Giebultowicz, J. M. and Riddiford, L. M. Role of the bursa copulatrix in egg maturation and reproductive behaviour of the tobacco hawk moth Manduca sexta. Int. J. Invertebrate Repr. (in press).

Giebultowicz, J. M. and Denlinger, D. L. Identification of neurons innervating the ring gland of the flesh fly larva, Sarcophaga crassipalpis Macquant (Diptera: Sarcophagidae). Int. J. Invertebrate Morphol. (in press).

Giebultowicz, J. M. and Denlinger, D. L. Role of the brain and ring gland in regulation of pupal diapause in the flesh fly, Sarcophaga crassipalpis. (in preparation)

1. Scientist's name, address, and telephone number:

Thomas J. Kelly
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2. Location:

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Insect Reproduction Laboratory
Beltsville, MD 20705

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

1.0

6. Technical capability and equipment:

HPLC and ecdysteroid RIA capabilities (with Chuck Woods) including liquid scintillation counters (Beckman LS-100C and Beckman LS-3801 with RIA data reduction package), Beckman TJ-6 low speed centrifuge and Spectra-Physics SP8700 solvent delivery system and high-pressure pump. (continued on next sheet)

7. Objectives of research:

Develop basic knowledge about the endocrine/neuroendocrine regulatory mechanisms governing insect reproduction and development. Develop and utilize biological, biochemical, analytical, immunological and genetic assays to identify, purify and characterize new hormones and/or neurohormones. Elucidate the mechanisms and regulation of synthesis, transport, metabolism and action on target tissues by these endogenous factors. Cooperate as a team member in developing and determining the function of exogenous hormone analogs, antagonists or suppressors that disrupt reproduction and development. This information will ultimately lead to the development of pest control methods based on reproduction-regulating processes.

Monoclonal and immunological capabilities (equipment shared with Edward P. Masler): Titertek ELISA reader, CO_2 incubator, inverted microscope, laminar flow hood, liquid nitrogen storage tank and immunoelectrophoresis equipment.

Immunocytochemistry and cobalt backfilling (with Yaga Giebultowicz).

Protein separation and purification capabilities (equipment shared with Edward P. Masler): horizontal and vertical slab gel electrophoresis, isoelectric focusing, gel scanner, low pressure chromatography and accessories.

Other equipment: Model L ultracentrifuge, Cahn electrobalance, Wild M-8 dissecting scope with photographic capabilities and fiber optics, American Optical Phase-Star compound scope with photographic capabilities.

8. Research priorities in your program:

Purify and characterize oostatic hormone from flies and mosquitoes, develop monoclonal antibodies, assays and methods for localizing sites of synthesis, mechanisms of release and transport, and mode of action. Cooperate on similar studies with egg development neurosecretory hormone. Cooperate in comparative studies on these hormones and in determining their interactions with juvenile hormones, ecdysteroids and biogenic amines. Demonstrate a role for makisterone A in egg development in true bugs.

9. Progress of current research in solving problems:

In vitro and in vivo bioassays have been developed for oostatic hormone, partial purification achieved by HPLC, and a new mode of action demonstrated. Ovarian ecdysteroid synthesis has been correlated with vitellogenesis in the autogenous mosquito, Aedes atropalpus, and the fruit fly, Drosophila melanogaster and some of the interactions with juvenile hormone demonstrated. Biogenic amines were ruled out as probable candidates for oostatic hormone. Biological cross-reactivity could not be demonstrated between Manduca sexta prothoracicotropic hormone and Aedes aegypti egg development neurosecretory hormone.

10. Significant research accomplishments in the past 3 years:

In cooperation with other scientists: (1) Demonstrated that makisterone A is the molting hormone of larval Oncopeltus fasciatus and apparently plant-feeding Hemiptera (true bugs). Previously thought to be 20-hydroxyecdysone in all insects and other arthropods.

(2) Demonstrated that the ovaries of Drosophila melanogaster are the major source of ecdysteroids in adult females and that they are secreted in vitro. Requires reevaluation of present theories about the regulation of vitellogenesis and egg maturation in Drosophila.

(3) Demonstrated a new mode of action for oostatic hormone in flies and mosquitoes. Oostatic hormone blocks the action of egg development neurosecretory hormone in vivo.

(4) Demonstrated that a molt inhibiting compound in Oncopeltus fasciatus prevented normal ecdysteroid production suggesting a novel mode of action for this class of compounds.

11. Impact of research accomplishments on science and the general public:

The basic knowledge derived from these accomplishments has helped to demonstrate and clarify the complexity and diversity of insect endocrine systems, once thought to be relatively simple by contrast to vertebrates. New concepts have been developed about the regulation of insect development and reproduction and new theories postulated about the evolution of insect hormones. New methods and assays have been established to facilitate purification of these endocrine factors and to establish their roles in insect reproduction and development. Ultimately, new and selective methods of pest control will be devised based in part on this knowledge.

12. Obstacles to achieving objectives:

(1) Difficulty in making major, and sometimes even minor, changes in research direction. Modifications of facilities are extremely slow and inadequately correlated with ongoing research resulting in unnecessary delays.

(2) Difficulty in obtaining funding and positions for intermediate level personnel, such as postdoctoral associates, which are necessary for a rapid influx of new techniques and ideas.

(3) Insufficient interaction with major universities for obtaining graduate student support.

(4) Too much time spent on procurement of fair and lowest price equipment resulting in unnecessary delays of research.

(5) Travel ceilings (monetary and personnel) that interfere with travel to scientific meetings (especially international) necessary for maintaining an international standing and lead in the field of expertise.

13. Future lines of needed research and plans for implementation

The goal of elucidating the endocrinological mechanisms regulating insect reproduction and development necessitates that all available techniques be utilized. Genetic and molecular genetic analyses are potent tools for clarifying the complex interactions involved in endocrine/neuroendocrine regulation. Unfortunately, attempts to utilize genetic mutants to unravel these interactions have been complicated by the availability of only minute amounts of endocrinological material, assays which are barely sensitive enough to detect these hormones, and insufficient knowledge of the correlations between endocrinologically and physiological, biochemical and morphological events, especially in Drosophila melanogaster, the most completely analyzed system in insects. Furthermore, the role of neurohormones such as egg development neurosecretory hormone, have not been adequately defined for Drosophila reproduction. Plans for implementation in this area include

13. continued

continuing to correlate endocrinological changes with developmental and reproductive events, to demonstrate a role for egg development neurosecretory hormone and oostatic hormone in Drosophila, to develop mutant strains containing abnormal levels of these hormones, to isolate and characterize these hormones and their structural and regulatory genes, to utilize molecular genetic analyses for elucidating their role in insect reproduction, and to develop selective methods for interfering with the normal synthesis and/or action of these hormones.

14. Research facilities and personnel needs:

Research facilities are adequate at present but space requirements may increase as programs solidify.

More intermediate level personnel such as postdoctoral associates and graduate students are required.

15. Extent of cooperation--names of persons and institutions:

Dr. Edward P. Masler
Insect Reproduction Lab.
B-306, R. 309
Beltsville, MD

Mr. Edward Dougherty
Insect Pathology Lab.
B-011A, R-231
Beltsville, MD

Dr. Charles Woods
Insect Reproduction Lab.
B-306, R-113
Beltsville, MD

Dr. Terrence Adams
Metabolism & Radiation Res. Lab.
State University Station
Fargo, ND

Mr. Albert DeMilo
Insect Reproduction Lab.
B-306, R-112
Beltsville, MD

Dr. Thomas Coudron
Biological Control of Insects
Res. Lab.
Columbia, MO

Dr. Richard Imberski
Department of Zoology
University of Maryland
College Park, MD

Dr. Walter Bollenbacher
Dr. LaVern Whisenton
Department of Biology, Univ. of NC
Chapel Hill, NC

16. Titles of publications for the last 3 years:

Kelly, T.J., Fuchs, M.S. and Kang, S.-H. Induction of ovarian development in autogenous Aedes atropalpus by juvenile hormone and 20-hydroxyecdysone. Internatl. J. Invert. Reprod. 3:101-112. 1981.

Kelly, T.J., Woods, C.W., Redfern, R.E. and Borkovec, A.B. Makisterone A: the molting hormone of larvael Oncopeltus? J. Exp. Zool. 218:127-132. 1981.

Patterson, J.L., Kelly, T.J. and Duman, J.G. Purification and composition of a thermal hysteresis producing protein from the milkweed bug, Oncopeltus fasciatus. J. Comp. Physiol. 142:539-542. 1981.

Redfern, R.E., Kelly, T.J. and Hayes, D.K. Comparison of ecdysteroid concentration in and growth regulating effects on 5th-stage Oncopeltus fasciatus (Dallas) treated with four insect growth regulators. In Juvenile Hormone Biochemistry: Action, Agonism, and Antagonism, Pratt, G.E. and Brooks, G.T. (Eds.), Elsevier/North Holland Biomedical Press, Amsterdam. pp. 415-420. 1981.

15. continued

Dr. Elaine Rubenstein
Department of Biology
Skidmore College
Saratoga Springs, NY

Dr. Barbara Stay
Dr. Susan Rankin
Department of Zoology
University of Iowa
Iowa City, IA

Dr. Denis Horn
CSIRO
Applied Chemistry Laboratories
Melbourne, Victoria
Australia

Dr. Shalom Applebaum
Department of Entomology
Faculty of Agriculture
The Hebrew University of Jerusalem
Rehovot, Israel

16. continue

Fuchs, M.S., Kang, S.-H., Kelly, T J., Masler, E.P. and Whisenton, L.R. Endocrine control of ovarian development in an autogenous mosquito. In Regulation of Insect Development and Behavior, Sehnal, F., Zabza, A, Menn, J.J. and Cymborowski, B. (Eds.), Wroclaw Technical University Press, Wroclaw, Poland. pp. 569-590. 1981.

Kelly, T J. and Hunt, L.M. Endocrine influence upon the development of vitellogenic competency in Oncopeltus. J. Insect Physiol. 28:935-941. 1982.

Kelly, T.J., Redfern, R.E., DeMilo, A.B. and Borkovec, A.B. Inhibition of ecdysis in Oncopeltus fasciatus by 2-acetylpyridine thiosemicarbazones. Pest. Biochem. Physiol. 17:35-41. 1982.

Aldrich, J.R., Kelly, T.J. and Woods, C.W. Larval molting hormones of tricophoran Hemiptera-Heteroptera: makisterone A not 20-hydroxyecdysone. J. Insect Physiol. 28:857-861. 1982.

Redfern, R.E., Kelly, T J., Borkovec, A.B. and Hayes, D.K. Ecdysteroid titers and molting aberrations in last-stage Oncopeltus nymphs treated with insect growth regulators. J. Pest. Biochem. Physiol. 18:351-356. 1982.

Rubenstein, E.B., Kelly, T.J., Schwartz, M.B. and Woods, C.W. In vitro synthesis and secretion of ecdysteroids by Drosophila melanogaster ovaries. J. Exp. Zool. 223:305-308. 1982.

Kelly, T.J., Aldrich, J.R., Woods, C.W. and Borkovec, A.B. Makisterone A: its distribution and physiological role as the molting hormone of true bugs. Experientia 40:996-997. 1984.

Kelly, T.J., Birnbaum, M.J., Woods, C.W. and Borkovec, A.B. Effects of house fly oostatic hormone on egg development neurosecretory hormone action in Aedes atropalpus. J. Exp. Zool. 229:491-496. 1984.

Kelly, T J., Woods, C.W., Birnbaum, M.J. and Borkovec, A.B. Oostatic hormone and biogenic amines as inhibitors of ovarian maturation in house flies and mosquitoes. In Insect Neurochemistry and Neurophysiology, Borkovec, A.B. and Kelly, T.J. (Eds.), Plenum Press, New York. pp. 401-403. 1984.

Birnbaum, M.J., Kelly, T J., Woods, C.W. and Imberski, R.B. Hormonal regulation of ovarian ecdysteroid production in the autogenous mosquito, Aedes atropalpus. Gen. Comp. Endocrinol. 56:9-18. 1984.

Borkovec, A.B. and Kelly, T.J., editors. Insect Neurochemistry and Neurophysiology, Plenum Press, New York. 1984.

Schnee, M.E., Ma, M. and Kelly, T J. Hormonal basis of gypsy moth (Lepidoptera: Lymantriidae) adult eclosion. J. Insect Physiol. 30:351-356. 1984.

Schwartz, M.B., Imberski, R.B. and Kelly, T J. Analysis of metamorphosis in Drosophila melanogaster: characterization of giant, an ecdysteroid deficient mutant. Develop. Biol. 103:85-95. 1984.

Ma, M., Newton, P.B., Gong, H., Kelly, T.J., Hsu, H.T., Masler, E.P. and Borkovec, A.B. Development of monoclonal antibodies for monitoring Aedes atropalpus vitellogenesis. J. Insect. Physiol. 30:529-536. 1984.

1. Scientist's name, address, and telephone number:

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2. Location:

Insect Reproduction Laboratory
Beltsville, MD

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's: 0.8

6. Objectives of research:

Physiological and endocrine regulation of the testes of the tobacco budworm, Heliothis virescens

a) Determination of somatic and neurosecretory hormones affecting the testes, and their roles in controlling sperm development
b) Determination of the roles of somatic and neurosecretory hormones in regulating the intrinsic secretion of ecdysteroid by the testes of this moth
c) Determination of the roles of the various ecdysteroids released by the testes
d) Study of the developmentally-linked changes in membrane junctions between the cells which envelop clones of developing sperm and the relation of this phenomenon to sperm development, sperm degeneration in diapause, and changes in hormone titers.

6a. a) Tissue culture: Laminar flow hood, sterilization equipment, inverted research microscope

b) Histology laboratory for whole mount, wax and cryo-sectioning, standard staining, and immunocytology

c) HPLC capability by cooperating scientists

d) Low power dissecting microscope with CO_2 anaesthesia chamber and foot focussing

e) RIA for ecdysteroids as routine procedure

f) JH esterase assay on line

7. Research priorities in your program:

All of the above

8. Progress of current research in solving problems:

a) Tissue culture studies are in progress to determine central nervous system factor effects on spermatocysts in controlled environments
b) A factor from brains of larvae and pupae of known developmental stages will induce production of ecdysteroid by immature testes. We are examining physical and chemical properties of the factor in crude preparations
c) Feedback loops are essential to neuroendocrine regulation in vertebrates; we are testing the effects of testis steroids in local (testicular) regulation in vito
d) We have established that different types of junctions can be found at distinct sperm developmental stages. We are investigating the relationship of the junctions to permeability of the spermatocysts in the presence of somatic and neurosecretory hormones

9. Significant research accomplishments in the past 3 years:

In Heliothis virescens, a)Brain factors are capable of increasing the rate of spermatogenesis, while subaesophageal ganglion factors inhibit spermatogenesis in vivo; b) Testes secrete ecdysteroid into surrounding fluid in vitro at specific times during development; c) Brain factors are capable of initiating ecdysteroid secretion by 'immature' testes, in vitro

10. Impact of research accomplishments on science and the general public:

a) Impact on Science: We have demonstrated that sperm maturation and ecdysteroid production by testes of H. virescens are under apparent neurohormonal control. Thus regulation of both male and female germ cells in insects is comparable to regulation in other animal groups and can be studied with available experimental tools.

b) Impact on the General Public: An increase in the understanding of insect reproductive physiology will lead to better control of insect pests by regulation of their fertility.

11. Obstacles to achieving objectives:

a) Lability of neural factors makes them difficult to handle and store.

b) Difficulty in rearing experimental animals in uniform quantities throughout the year makes performance of experiments ad libitum difficult.

12. Future lines of needed research and plan for implementation:

In future, neural factors should be isolated and characterized. Sufficient volumes of relatively pure factors should be readied for antibody production. Once antibodies are in hand, cytoimmunology to detect anatomical sites of synthesis and sites of receptors can be attempted. Cellular mechanisms of action can be studied.

13. Research facilities and personnel needs:

An eager student or two might help our program along. Space for these people, as well as incidental equipment and funds for their support will be needed.

14. Extent of cooperation--names of persons and institutions:

Cooperators: Staff of IRL
Dr. J. Giebultowicz, U. Maryland (College Park, MD) and IRL, USDA, Beltsville, MD
H. Jaffe, LIL/AEQI, BARC
J. Riemann, Radiation & Metabolism Res. Lab., Fargo, ND
K. Baldwin, Dept. of Anatomy, Howard Univ., Washington, DC

15. Titles of publications for the last 3 years:

See attached sheet

Publications:

1. Loeb, M.J. and Hayes, D.K. Neurosecretion during diapause and diapause development in brains of mature embryos of the Gypsy Moth, Lymantria dispar. Ann. Entomol. Soc. Amer. 73:432-436. 1980.

2. Loeb, M.J., Birnbaum, M.J. and Rochford, R. Spermatogenesis in the tobacco budworm, Heliothis virescens: The effect of haemolymph osmotic pressure. Amer. Zool. 20:945. Abstract. 1980.

3. Loeb, M.J. and Hayes, D.K. Critical periods in the regulation of the pupal molt of the tobacco budworm, Heliothis virescens. Ann. Entomol. Soc. Amer. 73:679-682. 1980.

4. Loeb, M.J. and Hayes, D.K. Strobilation in the Chesapeake Bay sea nettle Chrysaora quinquecirrha. V. Neurons and neurosecretion. Trans. Amer. Microsc. Soc. 100:264-270. 1981.

5. Loeb, M.J. and Birnbaum, M.J. The relationship of hemolymph osmotic pressure to spermatogenesis in the tobacco budworm, Heliothis virescens. Int. J. Invert. Reproduction 4:67-79. 1981.

6. Loeb, M.J. and Hayes, D.K. Heliothis virescens: Sensitivity of larvae to pyridoxine deficiency. Ann. Entomol. Soc. Amer. 74:623-625. 1981.

7. Jaffe, H., Loeb, M.J., Borkovec, A.B. and Hayes, D.K. Isolation of peptides from invertebrate nerve tissue by HPLC. Fed. Proc. 40:1643. Abstract. 1981.

8. Loeb, M.J. Diapause and development in the tobacco budworm, Heliothis virescens: A comparison of hemolymph ecdysteroid titers. J. Ins. Physiol. 28:667-673. 1982.

9. Masler, E.P., Loeb, M.J. and Woods, C.W. Ecdysteroid titer in the head of an anautogenous mosquito. Amer. Zool. 22:956. 1982.

10. Loeb, M.J., Woods, C.W., Brandt, E.P. and Borkovec, A.B. Larval testes of the tobacco budworm: A new source of insect ecdysteroids. Science 218:896-898. 1982.

11. Jaffe, H., Loeb, M.J., Hayes, D.K. and Holston, N. Rapid isolation of nanogram amounts of crustacean erythrophore concentrating hormone from invertebrate nerve tissue by RP-HPLC. J. Liquid Chromatog. 5:1375-1390. 1982.

12. Loeb, M.J., Brandt, E.P. and Masler, E.P. Control of spermatogenesis in the tobacco budworm, Heliothis virescens. The Southwestern Entomologist Supplement 5:33. 1983.

13. Loeb, M.J. and Dodson, J. Brain neurosecretion during development of the last larval instars of the tobacco budworm, Heliothis virescens. Trans. Am. Microsc. Soc. 103:44-57. 1984.

14. Loeb, M.J., Brandt, E.P. and Birnbaum, M.J. Ecdysteroid production by testes of the tobacco budworm, Heliothis virescens, from last larval instar to adult. J. Insect Physiol. 30:375-381. 1984.

15. Loeb, M.J. and Dodson, J. Progressive changes in stainable neurosecretion in brains of Heliothis virescens throughout the last larval instar. In Insect Neurochemistry and Neurophysiology, Borkovec, A.B. and Kelly, T.J. (Eds.), Plenum Publ. Corp., New York. 1984.

16. Jaffe, H., Loeb, M.J. and Hayes, D.K. Isolation of crustacean erythrophore-concentrating hormone from nerve tissue of Homarus americanus. Comp. Biochem. Physiol. In press.

17. Loeb, M.J., Brandt, E.P. and Woods, C.W. Ecdysteroid synthesis by testes of the tobacco budworm, Heliothis virescens, cultured in vitro. Proceedings of the sixth International Conf. on Invertebrate Tissue Culture. In Press.

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2. Location:

Northeast Region

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
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2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

1

6. Objectives of research:

The overall objective of the research is to develop a basic knowledge of the biochemistry and molecular biology which underlie the physiological mechanisms controlling insect reproduction. More specifically, the objectives include the isolation and characterization of neuropeptides, neurohormones and related factors, the elucidation of their roles in insect reproductive physiology and the determination of the mechanism of biosynthesis, processing and storage of these molecules and their modes of action.

6.a Expertise and facilities available

Training and expertise: developmental biology, cell biology, insect physiology, genetics, biochemistry, molecular biology, immunological assays and antibody produciton.
Facilities and experience: peptide and protein separation and analysis; peptide micromethods; LC, HPLC, electrophoresis, IEF, immunoelectrophoresis; monoclonal and polyclonal antibody production; radioimmunoassay, ELISA; cell and organ culture; ultracentrifugation (density gradient, both rate zonal and isopycnic); tissue extraction, etc.

7. Research priorities in your program:

The research program focuses on the molecular biology of female reproductive physiology, and the role of the brain and nervous system in the hormonal control of ovarian maturation. Of highest priority is the isolation and characterization of mosquito EDNH (egg development neurosecretory hormone), evaluation of its role in ecdysteroid production and ovarian development and production of monoclonal antibody to EDNH. In addition, essential efforts are directed toward the discovery of PTTH (prothoracicotropic hormone) activity in mosquitos and EDNH/PTTH activities in other insects; the discovery of potential endogenous inhibitors of these hormones and of ecdysteroid production; the development of appropriate bioassays; the development of analytical methods including protein and peptide isolation and modification, cell culture methods, hybridoma and immunochemical procedures.

8. Progress of current research in solving problems:

A methodology was developed for the isolation of EDNH and other bioactive peptides combining low and high pressure chromatographic procedures with various bioassays (in vitro and in vivo). A system was developed for the detection of endogenous inhibitors of ecdysteroid production and ovarian development. A program was initiated for the production of monoclonal antibodies against EDNH and other neuropeptides. The program includes cell culture and hybridoma techniques, analytical immunoassays such as RIA and ELISA, and novel bioassays.

9. Significant research accomplishments in the past 3 years:

Mosquito EDNH has been partially purified and characterized biochemically. Multiple forms of the neurohormone have been found and may have differential bioactivities. Antibody has been raised against EDNH fractions obtained by HPLC fractionation and an enzyme linked immunosorbent assay (ELISA) has been developed for the evaluation of antisera and detection of antigen EDNH. A cell culture-hybridoma program has been developed for the production of monoclonal antibody to EDNH and other neuropeptides. An endogenous inhibitor of ecdysteroid production, possibly an antogonist of EDNH, has been prepared from mosquito heads and partially characterized. EDNH activity has been identified in non-mosquito species and has chromatographic properties similar to mosquito EDNH. Significant ecdysteroid has been found in mosquito (Aedes aegypti) heads and found to have a variable titer regulated independent of the whole body titer and temporally related to EDNH secretion. The head was shown to be capable of metabolizing ^{3}H-ecdysone suggesting a regulatory mechanism within the head, coupling ecdysteroid feedback with neurosecretion.

10. Impact of research accomplishments on science and the general public:

The research accomplishments and approaches provide basic knowledge about molecular mechanisms involved in the control of insect reproduction. Results and discoveries serve to expand the base of fundamental information from which novel and enhanced approaches to insect pest control will arise. For example, work on EDNH isolation and characterization has stimulated similar investigations in other laboratories and generated significant interest in the scientific community. Results of studies on inhibitors of ecdysteroid production and ovarian development and the interaction of such inhibitors with stimulating factors such as EDNH, necessarily lead to reevaluation of our current understanding of the mechanisms of control of ovarian development. Studies at the basic level, then, accommodate an interdisciplinery approach which is necessary for the efficient use of research resources toward the ultimate goal of effective pest control.

11. Obstacles to achieving objectives:

There is an acute shortage of highly motivated and trained personnel, such as postdoctoral associates and graduate students, versed in the biochemical and molecular sciences. Such personnel not only provide needed expertise, but are a source of innovative ideas and state-of-the-art approaches. There is also a shortage of mid-level support scientists (GS6-9) capable of performing the sophisticated and demanding procedures required in this highly technological research. Clearly, sufficient funds are required for support of these highly trained professionals.

12. Future lines of needed research and plan for implementation:

Neuropeptide isolation and manipulation will be continued and expanded. The discovery of EDNH-like activity in a number of mosquito species and non-mosquito Diptera has begun and the evaluation of these systems in a comparative manner will be emphasized. Genetic studies designed to determine the relationship between the mechanism of EDNH release and type of mosquito ovarian development (autogenous v. anautogenous) have been initiated and will be continued as personnel conditions allow. The monoclonal antibody - hybridoma system will be more heavily used for neurohormone isolation, subcellular localization of neuropeptides, and studies on the synthesis, processing, transport and storage of peptide hormones. We also anticipate the use of molecular genetics and recombinant DNA technology to engineer EDNH producing cells for the production of amounts of peptide that will facilitate studies on hormonal regulatory mechanisms. A postdoctoral associate is sought for this project.

13. Research facilities and personnel needs:

Personnel: Postdoctoral associates in biochemistry and molecular biology
Mid-level support scientist for protein chemistry and peptide manipulati
Funds for graduate students in these areas

Facilities: Capital equipment such as an ultracentrifuge, FPLC protein chromatography system and personal computing facilities for data processing, manuscript preparation, literature searches and research program organization.

14. Extent of cooperation--names of persons and institutions:

T. S. Adams, ARS/MRRL, Fargo, ND
S. Applebaum, Hebrew University, Rehovot, Israel
W. Bollenbacher, University of North Carolina
D. Bolt, ARS-Reproduction Laboratory
A. B. DeMilo, IRL
E. Dougherty, ARS-Insect Pathology Laboratory, Beltsville, MD
A. Guidry, ARS-MSML, Beltsville, MD
J. Giebultowicz, IRL
T. J. Kelly, IRL
R. Lawson, ARS-FNCL, Beltsville, MD
M. Loeb, IRL
J. Neal, ARS-FNCL, Beltsville, MD
S. Rankin, University of Iowa
R. Rao, University of West Florida
B. Stay, University of Iowa
H.-T. Tsu, American Type Culture Collection, Rockville, MD
L. Whisenton, University of North Carolina
C. Woods, IRL

15. Titles of publications for the last 3 years:

1984-Preparation of egg development neurosecretory hormone using reverse-phase liquid chromatographic techniques. Proc. ICINN, Plenum Press.

Physiologically active factors in head extracts of the mosquito Aedes aegypti. Proc. ICINN, Plenum Press.

Utilization of monoclonal antibodies for mosquito vitellogenesis research. American Zoologist.

Development of monoclonal antibodies for monitoring Aedes atropalpus vitellogenesis. J. Insect Physiology

Modulation of the rate of spermatogenesis by the tobacco budworm, Heliothis virescens. Int. J. of Invert. Reproduction and Development.

1983-Partial purification of egg development neurosecretory hormone with reverse-phase liquid chromatographic techniques. Life Sciences.

Chymotrypsin and trypsin levels in adult Aedes atropalpus and Toxorhynchites brevipalpis. Comp. Biochem. Physiol.

Page 15 continued:

1983- Factors affecting ovarian maturation in two Aedes mosquitos. The Southwestern Entomologist.

Modulation of sperm development in the moth, Heliothis virescens, by the brain, subaesophageal ganglion, and interconnecting nerves. Third Int'l. Symp. of Invert. Reproduction.

Control of spermatogenesis in the tobacco budworm, Heliothis virescens. The Southwestern Entomologist.

1982- Biochemical properties of the two forms of Leucophaea maderae vitellin and their subunits. Insect Biochem.

Ecdysteroid titers in the head of an anautogenous mosquito. American Zoologist.

1. Scientist's name, address, and telephone number:

Charles W. Woods
Rm. 113, Bldg. 306, BARC-East
Beltsville, MD 20705
(301) 344-2924

2. Location:

Beltsville Agricultural Research Center

3. Number and title of CRIS work unit:

1203-20255-007 Characterization of insect neurohormones and related factors
1203-20255-008 Mechanism of hormonal regulation of insect reproduction

4. Approach Element and Problem Definitions:

2.4.01.2.a Hormone action, secretion, and metabolism: regulation of membrane function, enzyme activity, and control of homeostasis, development and reproduction
2.4.01.2.c Insect neurobiology: identification of regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation

5. Estimated SY's:

1.0

6. Objectives of research:

The isolation, characterization, and identification of insect hormones, particularly neuropeptides, that influence insect reproduction.

Available equipment includes HPLC, column packing equipment, gas chromatographs, and a mass spectrometer with direct insertion probe.

7. Research priorities in your program:

Development of HPLC and mass spectrograph procedures for the isolation and identification of insect hormones.

8. Progress of current research in solving problems:

9. Significant research accomplishments in the past 3 years:

Developed HPLC and MS methods which allowed isolation and identification of makisterone A as the molting hormone in larval Oncopeltus and several other insects.

Partially purified an ovarian maturation inhibitor by RP-HPLC.

Identified ecdysteroids produced by tobacco budworm testes.

10. Impact of research accomplishments on science and the general public:

Identification of makisterone A as a molting hormone refuted a long held belief that 20-hydroxyecdysone is the universal molting hormone.

11. Obstacles to achieving objectives:

Rearing problems require working with picagram quantities of active material. This also requires bioassays since physical detection methods are insufficiently sensitive.

12. Future lines of needed research and plan for implementation:

Rigid, non-silica based packing with a variety of coatings is becoming available and should be evaluated for hormone separations. Use over greater pH ranges, different selectivities, and higher recoveries may be obtained. Electrochemical detectors are being found useful at picagram levels for peptide derivatives and should be evaluated.

13. Research facilities and personnel needs:

Our 2 Waters HPLC units are 8 and 11 years old and while still adequate will eventually need replacement. A capillary column GC is needed for preliminary runs to establish conditions before injection into the GC-MS system.

A fast atom bombardment probe for the MS will be required for non-volatile samples.

14. Extent of cooperation--names of persons and institutions:

T. J. Kelly, IRL
D. Gelman, IRL
M. Loeb, IRL
H. Jaffe, LIL
R. Hakim, Howard University
M. Ma, University of Maryland
E. Rubenstein, Skidmore College

15. Titles of publications for the last 3 years:

Borkovec, A. B., and C. W. Woods, 1982. Alkylating agents. In Handbook of Carcinogens and Hazardous Substances, M. C. Bowman (Ed.), Marcel Dekker, New York, 1982, 750 pp., Chapter 2, pp. 19-74.

Gelman, D. B., C. W. Woods, and L. A. Brents. 1982. Haemolymph ecdysteroid titres: Non-diapause versus diapause-bound fourth and fifth instars of the European corn borer, Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae). 54th Ann. Mtg. Eastern Branch Entomol. Soc. Amer., Hartford CT, September 1982. Abstracts, p. 40.

Aldrich, J. R., T. J. Kelly, and C. W. Woods. 1982. Larval moulting of trichophoran Hemiptera-Heteroptera: Makisterone A, not 20-hydroxyecdysone. J. Insect Physiol. 28:857-861.

Rubenstein, E. C., T. J. Kelly, M. B. Schwartz, and C. W. Woods. 1982. In vitro synthesis and secretion of ecdysteroids by Drosophila melanogaster ovaries. J. Exp. Zool. 223:305-308.

Loeb, M. J., C. W. Woods, E. P. Brandt, and A. B. Borkovec. 1982. Larval testes of the tobacco budworm: a new source of insect ecdysteroids. Science 218:896-898.

Kelly, T. J., C. W. Woods, M. J. Birnbaum, and A. B. Borkovec, 1982. Physiological function and partial purification of an ovarian maturation inhibitor from the housefly, Musca domestica. Joint Mtg. Entomol. Soc. Amer., Can. Ont., November 29-December 3, 1982, Toronto, Abstract 649, p. 121.

Masler, E. P., M. J. Loeb, and C. W. Woods. 1982. Ecdysteroid titers in the head of an anautogenous mosquito. Ann. Mtg. Amer. Soc. Zoologists, December 27-30, 1982, Louisville, Kentucky, Abstract 22, p. 256.

Kelly, T. J., J. R. Aldrich, and C. W. Woods. 1982. Makisterone A, ecdysone and 20-hydroxyecdysone in Hemiptera-Meteroptera. Ann. Mtg. Amer. Soc. Zoologists, December 27-30, 1982, Louisiville, Kentucky, Abstract 22, p. 257.

Kelly, T. J., C. W. Woods, and M. J. Birnbaum. 1983. Physiological function and partial purification of an ovarian maturation inhibitor from the housefly, Musca domestica. Southwest Entomol., Suppl. 5:36-37.

Gelman, D. B., and C. W. Woods. 1983. Haemolymph ecdysteroid titers of diapause- and nondiapause-bound fifth instars and pupae of the European corn borer, Ostrinia nubilalis (Hubner). Comp. Biochem. Physiol. 76A:367-375.

Gelman, D. B., and C. W. Woods. 1983. Distribution of ecdysteroids in hemolymph and testes of last instars, pupae and pharate adults of the European corn borer, Ostrinia nubilalis. Amer. Zool. Abstract 24:991.

Kelly, T. J., C. W. Woods, M. J. Birnbaum, and A. B. Borkovec. 1984. Oostatic hormone and biogenic amines as inhibitors of ovarian maturation in house flies and mosquitoes. In Insect Neurochemistry and Neurophysiology, A. B. Borkovec and T. J. Kelly (Eds.), Plenum Press, New York, pp. 401-403.

Kelly, T. J., M. J. Birnbaum, C. W. Woods, and A. B. Borkovec. 1984. Effects of house fly oostatic hormone on egg development neurosecretory hormone action in Aedes atropalpus. J. Exp. Zool. 229:491-496.

Loeb, M. J., E. Brandt, and C. W. Woods. 1984. Regulation of steroid synthesis in testes of the tobacco budworm moth. 3rd Marcus Singer Symposium on Developmental Biology, University of Connecticut, Storrs, CT. Abstract.

1. Scientist's name, address, and telephone number:

Dora K. Hayes
USDA, ARS, NER, BARC,
Livestock Insects Laboratory
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301-344-2474

2. Location: Beltsville Agricultural Research Center, Beltsville, Maryland

3. Number and title of CRIS work unit:

1103-20254-001 (to be updated to 1203-20254-005

4. Approach Element and Problem Definitions:

2.4.01.2.a
Objective: Reproductivity/quality—crops
Approach: Reduce losses-weeds, dis, insct, nem
Approach element: Biology - insects
Problem: Inadequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate knowledge of the secretaion and metabolism of hormones and their action on membranes and enzyme functions in insects impedes discovery and development of improved control principles.

2.4.01.2.b
Objective: Productivity/quality—crops
Approach: Reduce Losses-weeds/dis/insct/nem/
Element: Biology - insects
Problem: Adequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate information about the role of membranes in the regulation of cell function is a barrier to the discovery of improved control principles (Note: Many receptors are in membranes)

2.4.01.2.c
Objective: Productivity/quality—crops
Approach: Reduce losses-weeds, dis,insct,nem
Approach element: Biology - insects
Problem: Inadequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Discovery of improved control principles is impaired by inadequate knowledge of insect neurobiology, including regulatory neurochemicals, functional morphology, and intrinsic and extrinsic regulation.

(Note to Dr. Wright: The CRIS assigns the work to a and b but c is also applicable.)

5. Estimated SY's: 0.6

6. Objectives to research: (1) To discover a receptor for at least one neuropeptide and one biogenic amine, preferably an indolamine. (2) To discover how production of peptide hormones is influenced by biogenic amines. (3) Eventually - to determine genetic code of at least one peptide and to manipulate production of this material by the insect—if support becomes availble.

7. Research priorities in your program: (1) Determination of presence and rhythmic properties of at least one receptor for an insect neuropeptide or neurotransmitter. At present the target is the LAKH receptor because a tyr-1-LAKH is available and this can be iodinated with I-125 so that localization is possible using radiotracer technology. (2) Determination of chronobiological parameters for peptide and/or neurotransmitter secretion or lack thereof.

8. Progress of current research in solving problems: Current research has indicated: (a) Novel method for bioassay of peptide activity of the adult face fly by topical application to the adult pronotum 24 h or less after eclosion. (b) Presence of peptides in various insects heretofore not characterized. (c) Importance of thythmicity and timing in sampling for peptides. (d) Potential for causing meaningful mortality by peptide application. (e) Effectiveness of a neuropeptide, CECH, in causing reduction of fecundity and increased mortality in face fly larvae and adults.

9. Significant research accomplishments in the past 3 years: (a) Discovery (with H. Jaffe) of neuropeptides heretofore not noted in insect nervous tissue (this will be covered in detail by Dr. Jaffe's report). (b) Demonstration of a "putative" melatonin (i.e., R_f is similar on two hplc columns, but absorption ratios 214/280 and absorption fluorescent ratio (2/4/Fl) are not consistent. (c) Discovery of a 4.5 day rhythm in mortality of the face fly in response to treatment with placebo or the peptide, ACTH 1-17. Thus, a second, important control is available for comparison in bioassays. (d) Development of a simple hplc technique for measurement of melatonin in insect nervous tissue.

10. Impact of research accomplishments on science and the general public: This research has direct application to pest insect population management because it will provide the framework for novel insect control methodologies. These can include (a) New "Xth generation insecticides". (b) New, heretofore undeveloped techniques for population management. (c) Genetic modification of insects so that aberrant membrane proteins in pest insects do not function in binding hormones. (d) Application of findings on insects to human health and biology by application of new discoveries on receptors to human problems, including those of chronobiology.

11. Obstacles to achieving objectives: Obstacles to achieving objectives include: Lack of appropriately trained personnel, i.e. individuals with training in molecular biology, insect physiology and biochemistry and assistants with training as medical technologists; Lack (at the present time) of access to timely amino acid analysis, peptide sequencing, monoclonal antibody production and peptide synthesis.

12. Future lines of needed research and plan for implementation:

Needed research.

(a) Discover and develop information on nature and function of insect receptors, the binding sites for molecular messengers.

(b) Discover how molecular genetics can be applied to studies on receptors for insect peptide and biogenic amines.

(c) Maintain the biological rhythms (chronobiologic) viewpoint to maximize effective studies.

Plan for implementation.

(a) Demonstrate the presence of receptors for insect peptides, beginning with tyr-1-adipokinetic hormone labelled with I-135, in cooperation with Dr. T. August, Johns Hopkins Universtity.

(b) Utilize and further develop techniques learned in (a) to discover receptors for biogenic amines, specifically melatonin, or related compounds in insect membranes.

(c) Isolate and characterize receptors.

(d) Develop genetic information on receptors. An introduction to these techniques will be obtained during the time of study at Johns Hopkins.

(e) Determine if and how biogenic amines serve as molecular messengers whose action results in synthesis, degradation, or activation of receptors.

13. Research facilities and personnel needs: Research laboratory area is adequate. Equipment for running electrophoretic analyses and dot blot is needed. Radiotracer equipment should be upgraded. Lack of appropriately trained personnel, i.e., one molecular biologist, one insect physiologist, one biochemist and three technicians with training as medical technologists.

14. Extent of cooperation--names of persons and institutions:

Thomas August, Johns Hopkins University, Baltimore, Md. - 1984-85.
I. E. A. Gaaboub, University of Cairo, Egypt - 1983-84.
F. Halberg, University of Minnesota Medical School - 1983-continuing.
R. W. Miller, R. E. Redfern and L. Shade, LIL - 1984-continuing.
A. Raina, OCSL, Beltsville, Md. - 1984-continuing.

Dr. August has facilities for axenic tissue culture and for monoclonal antibody production. I attach a brief description of the work with Dr. August in the form of a memo to the BARC Area Director.

15. Titles of publications for the last 3 years:

See attached list.

Publications

Gelman, D. and Hayes, D. Critical periods for the brain and prothoracic glands of 5th instars of the European corn borer, Ostrinia nubilalis Hübner. Comp. Biochem. Physiol. 73A(1):81-89. 1982.

Gelman, D. B. and Hayes, D. K. Methods and markers for synchronizing maturation of 5th-instar larvae and pupae of the European corn borer, Ostrinia nubilalis (Lepidoptera: Pyralidae). Annals of the Entomol. Soc. of Am. 75(5):485-493. 1982.

Jaffe, Howard, Loeb, Marcia, Hayes, Dora K. and Holston, Nancy. Rapid isolation of nanogram amounts of crustacean erythrophore concentrating hormone from invertebrate nerve tissue by RP-HPLC. J. of Liquid Chromatography 5(7):1375-1390. 1982.

Redfern, R. E., Kelly, T. J., Borkovec, A. B. and Hayes, D. K. Ecdysteroid titers and molting aberrations in last-stage Oncopeltus nymphs treated with insect growth regulators. Pest. Biochem. Physiol. 18:351-356. 1982.

Hayes, D. K. Chronobiology of peptide hormones - possible applications to studies in insects. Proc. of a Workshop on Insect Neuropeptides, June 1982. College Station, Tx. Southwest. Entomol. Suppl. No. 5, p 13-15. 1983.

Hayes, D. K., Cornelissen, G., Halberg, F., Shankariah, K. Survival time of the face fly model as a gauge of circaseptan organization and optimization. Chronobiologia Vol. X(2): #64, p 132. 1983. (Abstract)

Hayes, D. K., Redfern, R. E., Schmidtmann, E. T. Diapause in the face fly, Musca autumnalis De Geer. Chronobiologia Vol. X(2): #65. 1983. (Abstract)

Jaffe, H. and Hayes, D. K. Analysis of invertebrate peptide hormones by RP-HPLC. J. Liq. Chromatography 6(6):993-1013. 1983.

Jaffe, H., Hayes, D. K., Sonenshine, D. E., Dees, W. H. and Thompson, M. J. Controlled release reservoir systems for the delivery of insect steroid analogues against ticks. Proc. 10th Intl. Symp. on Controlled Release of Bioactive Materials, July 1983, San Francisco, Calif., 67-70. 1983.

Jaffe, H., Rao, K. R., Hayes, D. K. and Garvick, S. Reverse-phase HPLC of crustacean chromatophorotropins from insect nervous tissue. Proc. 3rd Intl. Symp. on HPLC of Proteins, Peptides, and Polynucleotides, Monte Carlo, Monaco, No. 221. 1983. (Abstract)

Gaaboub, I. A. and Hayes, D. K. Biological activity of azadirachtin, a molting inhibitory component of the Neem tree, against the face fly, Musca autumnalis De Geer (Diptera:Muscidae). Environ. Entomol. 13:803-812. 1984.

Gelman, D. B. and Hayes, D. K. The influence of temperature on photo- periodic control of diapause induction in different strains of the European corn borer. In Insect Neurochemistry and Neurophysiology. (Alexej B. Borkovec and Thomas J. Kelly, eds.). Plenum Press, New York. p 357-359. 1984.

Hayes, Dora K. The history and present status of aircraft disinsection. In Commerce and the Spread of Pests and Disease Vectors, Praeger Pubs, New York. Proc. XV Pacific Science Congress, Symp. 7, Accidental Introduction of Insects through Human Agency, New Zealand (Dunedin), Feb. 83. p. 23-36. 1984.

Jaffe, H., Loeb, M., Hayes, D. K., Talbot, N. and Garvick, S. Isolation of crustacean erythrophore-concentrating hormone from nerve tissue of Homarus americanus. Comp. Biochem. Physiol. 78C(2):397-401. 1984.

Jaffe, H., Sonenshine, D. E., Hayes, D. K., Dees, W. H., Beveridge, M. and Thompson, M. J. Effects of the controlled release of ecdysteroids on the development and sex pheromone activity in ticks. Proc. 11th Intl. Symposium on Controlled Release of Bioactive Materials, July 23-25, 1984. Ft. Lauderdale, Fla., p. 118-119. 1984.

Gaaboub, I. A. and Hayes, D. K. Mortality rate and ovarian development of adults of the face fly, Musca autumnalis De Geer (Diptera:Muscidae) topically treated with the polypeptide neurohormone CECH. Environ. Entomol. Accepted for publication.

Gaaboub, I. A. and Hayes, D. K. The effect of larval treatment with Azadirachtin, a molting inhibitory component of neem tree, on reproductive capacity of face fly, Musca autumnalis De Geer (Diptera:Muscidae). Environ. Entomol. Accepted for publication.

Hayes, D. K. Biological rhythms and development of agricultural chemicals. Proceedings of Agricultural Chemicals of the Future (BARC Symposium No. 8, May 16-19, 1983, James L. Hilton, ed.). Rowman and Allanheld, Pubs. In press.

Hayes, D. K., Shade, L., Cornelissen, G., Halberg, E., Miller, R. W. and Halberg, F. Role for insects in chronobiologic technology transfer: Infradian synchronization by placebo or ACTH 1-17 of Musca vitripennis mortality on shifted light regimens. In the Proceedings of the International Workshop on Chronobiological Technologies. Como, Italy, 1984. (Accepted for publication pending USDA-Agr. Res. Serv. approval.)

Pickens, L. G. and Hayes, D. K. Evaluation of a new face fly and stable fly trap which segregates the catch of the two species. Environ. Entomol. Environ. Entomol. In Press.

1. Scientist's name, address, and telephone number:

Howard Jaffe
USDA, ARS, NER, BARC,
Livestock Insects Laboratory
Rm. 120, Bldg. 307, BARC-East
Beltsville, Maryland 20705
301-344-2474

2. Location: Beltsville Agricultural Research Center, Beltsville, Maryland

3. Number and title of CRIS work unit:

1103-20254-001 - Biochemical and Biophysical Studies on Insects Affecting Livestock

4. Approach Element and Problem Definitions:

2.4.01.2.a
Objective: Reproductivity/quality--crops
Approach: Reduce losses-weeds, dis, insct, nem
Approach element: Biology - insects
Problem: Inadequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate knowledge of the secretaion and metabolism of hormones and their action on membranes and enzyme functions in insects impedes discovery and development of improved control principles.

2.4.01.2.b
Objective: Productivity/quality--crops
Approach: Reduce Losses-weeds/dis/insct/nem/
Element: Biology - insects
Problem: Adequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate information about the role of membranes in the regulation of cell function is a barrier to the discovery of improved control principles (Note: Many receptors are in membranes)

5. Estimated SY's: 1.0

6. Objectives of research: Isolation, purification and identification of invertebrate peptides. In general our laboratory concentrates on hplc isolation and purification. Bioassay and structural determination are done by various cooperators.

7. Research priorities in your program: (a) Isolation and identification of pheromone activating neuropeptide(s) from Heliothis zea and H. virescens. (b) Isolation and identification of crustacean chromatophorotropic peptides from H. zea and H. virescens. (c) Isolation of crustacean chromatophorotropic peptides from brains of Diptera.

8. Progress of current research in solving problems: (a) Isolation and purification of pheromone activating neuropeptide hormone (PANH) from Heliothis zea and virescens. A peptide affecting the Z-11-C_{16}-aldehyde has been purified . (b) Isolation of crustacean chromatophorotropic peptides from H. zea and virescens. A peptide with weak chromatophorotropic activity has been purified and submitted for amino-acid analysis. (c) Isolation of crustacean chromatophorotropic peptides from brains of Musca autumnalis. Two activities, concentrating and dispersing, have been identified and separated.

9. Significant research accomplishments in the past 3 years: (a) Rapid isolation method for invertebrate peptides. Isolated and identified CECH in paleomenetes pugio eyestalks. (b) Identified CECH as first peptide from Homarus americanus. (c) Isolated and purified pheromone activating neuropeptide hormone (PANH) from Heliothis zea and virescens. A peptide affecting the Z-11-C_{16}-aldehyde has been purified. (d) Isolated crustacean chromatophorotropic peptides from H. zea and virescens. A peptide with weak chromatophorotropic activity has been purified and submitted for amino-acid analysis. (e) Isolated crustacean chromatophorotropic peptides from brains of Musca autumnalis. Two activities, concentrating and dispersing, have been identified and separated.

10. Impact of research accomplishments on science and the general public: The isolation and identification of insect peptides can lead to development of new, safe, methods of pest control.

11. Obstacles to achieving objectives: Additional support personnel, i.e. technicians, support scientist, 1040s.

12. Future lines of needed research and plan for implemenations:

    Future Plans. During the next year we hope to isolate and purify panh(s) and crustacean chromatophorotropic peptides from moth and fly heads (face fly and screwworm) and brains. A combination of the following techniques are to be utilized:

    (a) Preparative, analytical and microbore reverse-phase hplc.
    (b) High performance size-exclusion chromatography (hp-sec).
    (c) Variation of the bonded phase (e.g. C_{18}, C_8, wide-pore silica) and the ion-pairing buffer (e.g. TFA, TEAP).

    We anticipate being able to submit samples for amino-acid analysis and microsequencing. We also hope to work on the isolation of ovarian inhibition factors from diapausing face fly.

13. Research facilities and personnel needs Need additional support personnel to operate the new equipment presently being bought on-line to do the following: (a) Unattended method development and analysis. (b) Storage of data on disc. (c) Fluorescence detection. (d) Microbore gradient hplc.

14. Extent of cooperation--names of persons and institutions:

A. Raina, OCSL, Beltsville, Md.
R. Rao, U. of West Florida, Pensacola, Fla.
R. Wagner, Vet. Tox. & Ent. Lab., College Station, Tx.
T. Adams, Insect Phys. & Metabolism Res., Fargo, N.D.
M. Loeb, IRL, Beltsville, Md.

15. Titles of publications for the last 3 years:

See attached list.

Jaffe, H., M. Loeb, D. K. Hayes, and N. Holston. Isolation of crustacean erythrophore concentrating hormone from nerve tissue of Homorus americanus. Proc. 2nd Intl. Symp. on HPLC of Proteins and Peptides, Baltimore, Md., No. 310-20. 1982. (Abstract)

Jaffe, H., M. Loeb, D. K. Hayes, and N. Holston. Rapid isolation of nanogram amounts of crustacean erythrophore concentrating hormones from invertebrate nerve tissues by RP-HPLC. J. Liq. Chromatography 5(7):1375-1390. 1982.

Jaffe, H. Crustacean erythrophore-concentrating hormone and locust adipokinetic hormone in crustacea and insects: detection, isolation and bioassay. Proc. of a Workshop on Insect Neuropeptides, June 1982, College Station, Tx. Southwest. Entomol. Suppl. No. 5, p. 16. 1983.

Jaffe, H. Development of controlled release implantable systems against arthropod pests of livestock. Proc. Beltsville Symposium VIII, Agricultural Chemicals of the Future, No. 13, 1983. (Abstract)

Jaffe, H. Separations of two invertebrate peptides by HPLC with a multi-channel high-speed spectrophotometric detector. Liquid Chromatography and HPLC Magazine. 1(8):418-426. 1983.

Jaffe, H. and D. K. Hayes. Analysis of invertebrate peptide hormones by RP-HPLC. J. Liq. Chromatography, 6(6):993-1013. 1983.

Jaffe, H., D. K. Hayes, D. E. Sonenshine, W. H. Dees, and M. J. Thompson. Controlled release reservoir systems for the delivery of insect steroid analogues against ticks. Proc. 10th Intl. Symp. on Controlled Release of Bioactive Materials, July 1983, San Francisco, Calif., 67-70. 1983.

Jaffe, H., K. R. Rao, D. K. Hayes, and S. Garvick. Reverse-phase HPLC of crustacean chromatophorotropins from insect nervous tissue. Proc. 3rd Intl. Symp. on HPLC of Proteins, Peptides, and Polynucleotides, Monte Carlo, Monaco, No. 221. 1983. (Abstract)

Jaffe, H. HPLC isolation of invertebrate neuropeptides. 1984 HPLC Symposium in the Separation of Biologically Active Molecules, June 14, 1984. (Abstract)

Jaffe, H., M. Loeb, D. K. Hayes, N. Talbot, and S. Garvick. Isolation of crustacean erythrophore-concentrating hormone from nerve tissue of Homarus americanus. Comp. Biochem. Physiol. 78C(2):397-401. 1984.

Jaffe, H., D. E. Sonenshine, D. K. Hayes, W. H. Dees, M. Beveridge and M. J. Thompson. Effects of the controlled release of ecdysteroids on the development of sex pheromone activity in ticks. Proc. 11th Intl. Symposium on Controlled Release of Bioactive Materials, July 23-25, 1984. Ft. Lauderdale, Fla., p. 118-119. 1984.

Feldmesser, J., J. Kochansky, H. Jaffe and D. Chitwood. Future Chemicals for control of nematodes. Proc. of Agricultural Chemicals of the Future (BARC Symposium No. 8, May 16-19, 1983, James L. Hilton, ed.). Rowman and Allanheld, Pubs. (In press)

1. Scientist's name, address, and telephone number:

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USDA, ARS, NER, BARC,
Livestock Insects Laboratory
Rm. 10, Bldg. 309, BARC-East
Beltsville, Maryland 20705
301-344-2298

2. Location: Beltsville Agricultural Research Center, Beltsville, Maryland

3. Number and title of CRIS work unit:

1103-20254-005 - Chemistry and Biochemistry of Peptide Neurohormones and Neurotransmitters Affecting Insect Development and Diapause.

4. Approach Element and Problem Definitions:

2.4.01.2.a
Objective: Reproductivity/quality--crops
Approach: Reduce losses-weeds, dis, insct, nem
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Problem: Inadequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate knowledge of the secretaion and metabolism of hormones and their action on membranes and enzyme functions in insects impedes discovery and development of improved control principles.

2.4.01.2.b
Objective: Productivity/quality--crops
Approach: Reduce Losses-weeds/dis/insct/nem/
Element: Biology - insects
Problem: Adequate basic knowledge of hormones and bioregulation of the life processes in insects impedes the discovery and exploitation of vulnerabilities in these processes as a means of control.
Subproblem: Inadequate information about the role of membranes in the regulation of cell function is a barrier to the discovery of improved control principles (Note: Many receptors are in membranes)

5. Estimated SY's: 35%

6. Objectives of research: Discover (develop) new and/or novel approaches for evaluation of activity of insect neuropeptides.

7. Research priorities in your program: Development of bioassays for evaluation of candidate peptide compounds.

8. Progress of current research in solving problems: The JH wax test has been modified to evaluate minute quantities of JH titers in hemolymph of diapausing and non-diapausing insects. It is hoped this method of presenting candidate compounds to test insects will work with peptides.

9. Significant research accomplishments in the past 3 years: Discovered photoperiod/temperature response required to induce diapause in the face fly.

   Discovered physical changes in the large milkweed bug which are instrumental (in joint work with IRL) in demonstrating new ecdysteroid activity.

10. Impact of research accomplishments on science and the general public: Based on requests for reprints, my research is having considerable impact on research. Very little of my research would be of immediate value to the general public.

11. Obstacles to achieving objectives: Need help with insect rearing, 1040 student, etc. and additional space for work with attractants, and/or repellents from narcotic plant research.

12. Future lines of needed research and plan for implementation: Additional research is needed to develop in vivo and in vitro bioassays for our ongoing peptide work. Plan to work on developing new bioassays as time permits.

13. Research facilities and personel needs:

    (1) 1040 student.
    (2) Large Room in Bldg. 309 - now occupied by John Ruth (Primarily storage).
    (3) 6'X6'X6' Screen Room - for large Room in 309.

14. Extent of cooperation--names of persons and institutions:

    Martin Jacobson, BANPL, AEQI
    Dave Warthen, Jr., BANPL, AEQI
    Novel Wakabayshi, BANPL, AEQI
    Dennis Vooden, BANPL, AEQI
    Terry McGovern, OCSL, AEQI
    A. B. DeMilo, IRL, AEQI
    Marsha Loeb, IRL, AEQI
    Dale Gelman, IRL, AEQI
    Philip Vincent, WSL, AEQI

15. Titles of publications for the last 3 years:

    See attached list.

Kelly, T. J., Redfern, R. E., DeMilo, A. B. and Borkovec, A. B. Inhibition of ecdysis in Oncopeltus fasciatus by 2-acetylpyridine thiosemicarbazones. Pest. Biochem. Physiol. 17:35-41. 1982.

Redfern, R. E., Kelly, T. J., Borkovec, A. B. and Hayes, D. K. Ecdysteroid titers and molting aberrations in last-state Oncopeltus nymphs treated with insect growth regulators. Pest. Biochem. Physiol. 18:351-356. 1982.

Stokes, J. B. and Redfern, R. E. Effects of sunlight on Azadirachtin: Antifeeding potency. J. Environ. Sci. and Health. A17(1):57-65. 1982.

Warthen, J. D. Jr., Redfern, R. E., Eubel, E. C. and Mills, G. D. Jr. Antifeedant screening of thirty-nine local plants with fall armyworm larvae. J. Environ. Sci. and Health. A17(6):885-895. 1982.

DeMilo, A. B., Redfern, R. E. and Borkovec, A. B. 2-acetylpyridine thiosemicarbazones as inhibitions of ecdysis in Oncopeltus fasciatus: Structure-activity relationship study. J. Agric. Food Chem. 31(4):713-718. 1983.

Hayes, D. K., Redfern, R. E., and Schmidtmann, E. T. Diapause in the face fly, Musca autumnalis De Geer. Chronobiologia Vol. X(2): #65. 1983. (Abstract)

Redfern, R. E., Warthen Jr., J. D., Jacobson, M., and Stokes, J. B. Anti-feeding potency of neem formulations. J. Environ. Sci. and Health. A19(4):477-481. 1984.

Jacobson, M., Stokes, J. B., Warthen Jr., J. D., Redfern, R. E., Reed, D. K., Webb, R. E., and Telek, L. Neem research in the U. S. Department of Agriculture: An update. Proc. 2nd International Neem Conference, May 1983. (Accepted for publication).

Redfern, R. E. Handbook of Naturally Occurring Pesticides, V. 1: Methods, Theory and Practice: Insect Bioassays. Ed. Dr. N. Bhushan Mandava. (In press) (Book Chapter).

Stokes, J. B., Redfern, R. E., Warthen Jr., J. D., Jacobson, M. Anti-feeding potency of neem formulations. Proc. Beltsville Symposium VIII, Agricultural Chemicals of the Future. 1983. (Abstract)

1. Scientists' name, address; and telephone number:

Ashok K. Raina and R. L. Ridgway
Organic Chemical Synthesis Laboratory, Bldg. 007, BARC-West,
Beltsville, MD 20705 and Dept. of Entomology, University of Maryland
344-4396

2. Location:

Beltsville, MD

3. Number and title of CRIS work unit:

1203-20256-001 Biosynthesis, emission and chemoreception of insect behavioral chemicals

4. Approach Element and Problem Definitions:

2.4.01.3d

5. Estimated SY's:

0.5 through cooperative agreement with University of Maryland
0.1 in-house

6. Objectives of research:

a. To isolate and purify sufficient amount of pheromone gland activating neurohormone (PANH).
b. To determine the amino acid composition and sequence of PANH.
c. To determine biological characteristics and mode of action of PANH.
d. To identify the source of PANH in brain (Neurosecretory cells).

7. Research priorities in your program:

Determine hormonal & other factors that control pheromone production in the female and their reception in the male.

8. Progress of current research in solving problems:

Knowledge of pheromone chemistry and pheromone-mediated male behavior is rapidly advancing. A brain hormone that controls pheromone production in Heliothis zea and perhaps in a number of other moths has been isolated. Similar activity has been detected in brains of several different species of insects.

9. Significant research accomplishments in the past 3 years:

a. Identification of sex pheromones of 4 species of moths, and study of pheromone-mediated male behaviors.
b. Eluidation of the diel periodicity of pheromone production and effect of age and mating on pheromone titer in Heliothis zea.
c. Discovery of PANH in Heliothis zea.

10. Impact of research accomplishments on science and the general public:

Knowledge of pheromone chemistry is utilized to formulate pheromone baits for use in traps and for mating disruption. A hormone that controls pheromone biosynthesis, once sequenced and synthesized, could be used to block this biosynthesis. The knowledge can also provide an insight into how an insect maintains a specific blend of its pheromone components.

11. Obstacles to achieving objectives:

None

12. Future lines of needed research and plan for implementation:

To determine if the same or similar hormone is present in other Lepidoptera. What role does it have in males? These aspects would be studied after the sequencing of PANH from H. zea is completed.

13. Research facilities and personnel needs:

Since we need a large number of Heliothis brains, technical help is essential to dissect these. Also need a biochemist to work full time on the mode of action of PANH.

14. Extent of cooperation--names of persons and institutions:

We are actively cooperating with Dr. Howard Jaffe of Livestock Insect Laboratory, ARS, Beltsville, towards isolation and purification of PANH and other peptide hormones. Once enough PANH is isolated we will be setting up collaboration with Veterinary Toxicology and Entomology Research Laboratory at College Station, TX, for amino acid analysis and sequencing of this hormone.

15. Titles of publications for the last 3 years:

Raina, A. K., and J. A. Klun. 1983. Neuro-hormonal control of sex pheromone production in Heliothis zea. Proc. Int. Conf. Insect Neurochemistry and Neurophysiology, Aug. 1-3, 1983. College Park, MD. A. B. Borkovec and T. J. Kelley, Eds., Plenum Press, New York. pp. 467-469.

Raina, A. K., and J. A. Klun. 1984. Brain factor control of sex pheromone production in the female corn earworm moth. Science 225: 531-533.

1. Scientist's Name, Address, and Telephone Number:

Dr. Douglas J. Bolt
USDA-ARS-NER-BARC
Animal Science Institute
Reproduction Laboratory
Bldg. 200, BARC-East
Beltsville, MD 20705

301/344-2529

2. Location:

Beltsville, MD

3. Number and Title of CRIS Work Unit:

1206-20354-001: Hormonal Mechanisms in Animal Reproduction

4. Approach Element and Problem Definitions:

3.2.01.1.a.

5. Estimated SY's:

.2

6. Objectives of Research:

To develop methods to investigate the mechanisms whereby inhibin, a glycoprotein produced by the ovary and found in ovarian follicular fluid, regulates the secretion of follicle-stimulating hormone, a pituitary hormone responsible for ovarian growth and development. The major obstacle to understanding these mechanisms is the lack of a sensitive, specific, and reliable assay for inhibin. Therefore, the first step will be to fractionate and purify sufficient inhibin to permit the development of an antiserum suitable for an RIA of inhibin.

7. Research Priorities in Your Program:

To improve reproductive efficiency of dairy cattle and swine.

8. Progress of Current Research in Solving Problems:

Large quantities of bovine and porcine follicular fluid have been obtained. The biological effects of these unfractionated preparations have been evaluated in swine, sheep and rats. Various fractionation strategies have been evaluated. Fast protein liquid chromatography appears to be the chromatographic approach of choice because it permits good recovery of biological activity and good separation of inhibin from contaminating peptides.

9. Significant Research Accomplishments in the Past 3 Years:

Until recently, reliable quantitative methods to investigate the endocrinology of FSH in farm animals were not available. Successful research at BARC has produced specific RIA's for ovine, porcine and bovine FSH and the reagents for these assays are now available through the USDA Animal Hormone Program. As a result, research in this country and around the world on the endocrinology of FSH in farm animals is proceeding at a much accelerated rate.

10. Impact of Research Accomplishments on Science and the General Public:

A major impact has been the development of specific assays for FSH in farm animals and the implementation of the USDA Animal Hormone Program, which makes these and other endocrine research reagents available. These efforts have provided the research tools and methodology that previously limited research to explore many of the key endocrine mechanisms that regulate reproductive efficiency in farm animals. As an example, in 1983, 76 U.S. and 17 foreign scientists were provided reagents and information essential for them to conduct research on FSH.

11. Obstacles to Achieving Objectives:

The major obstacles to achieving our research objectives are the same as for most research objectives, a limited amount of research manpower with limited time and resources using less than ideal scientific creativity. A specific obstacle for progress to explore endocrine mechanisms is the lack of reliable assays for the hormone involved. In some cases, certain scientific instruments with specific capabilities would accelerate progress.

12. Future Lines of Needed Research and Plan for Implementation:

Research to purify and develop a sensitive and reliable assay for inhibin will continue. Several bioactive factors that appear to exert important regulatory roles on the endocrine system have been demonstrated in the ovarian folliclular fluid. Research will be initiated to fractionate and purify some of these, including FSH-BI, a factor that modulates the effect of FSH by inhibiting the binding of FSH to its receptor located within the ovarian follicle.

13. Research Facilities and Personnel Needs:

Facilities are adequate. One additional SY and technical support would greatly accelerate research progress to isolate, purify, and investigate the endocrinology of bioactive regulators in the ovarian fluid.

14. Extent of cooperation--Names of Persons and Institutions:

L. E. Reichert, Jr., Albany Medical School, New York

G. H. Kiracofe, Kansas State University

15. Titles of Publications for the Last 3 Years:

Bolt, D. J. and R. Rollins. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone. J. Anim. Sci. 56:146-154. 1983.

Guthrie, H. D. and D. J. Bolt. Changes in plasma estrogen, luteinizing hormone, follicle-stimulating hormone and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ during blockade of luteolysis in pigs after human chorionic gonadotropin treatment. J. Anim. Sci. 57:993-1000. 1983.

Miller, K. F., D. J. Bolt and R. A. Goldsby. A rapid solution-phase screening technique for hybridoma culture supernatants using radiolabeled antigen and a solid-phase immunoadsorbent. J. Immunol. Methods 59:277-280. 1983.

Bolt, D. J. Development of a homologous radioimmunoassay for ovine follicle-stimulating hormone: Studies after estrus, ovariectomy, estradiol and releasing hormone. J. Anim. Sci. 53:730-741. 1981.

Bolt, D. J., R. M. Rollins and H. D. Guthrie. Development of a porcine FSH radioimmunoassay. J. Anim. Sci. 53 (Suppl. 1):298. 1981. (Abstract)

Kiracofe, G. H., J. A. Ramirez-Godinez, R. D. McGowan and D. J. Bolt. Reduction of serum FSH in ovariectomized heifers with bovine follicular fluid. J. Anim. Sci. 57 (Suppl. 1):350. 1983. (Abstract)

1. Scientist's name, address, and telephone number:

Robert A. Bell
ARS - USDA
Building 1398
Otis ANGB, MA 02542

Comm: (617) 563-9303
FTS: 840-7209

2. Location:

Otis Methods Development Center
Otis ANG Base, MA 02542

3. Number and title of CRIS work unit:

WRU# 1315-20251-001 Accession# 0043029
"Improve technology for lab culture, mass production and use of the gypsy moth and its natural enemies"

4. Approach Element and Problem Definitions:

2.4.1a.6 Develop fundamental understanding of nutrition and rearing in insects.

2.4.1a.17 Develop a comprehensive understanding of biological rhythms, diapause... in insects.

5. Estimated SY's:

2.4.1a.6 - 0.4 SY

2.4.1a.17 0.4 SY

6. Objectives of research: (Broad and Long Term)

(1) Develop fundamental principles involved in mass production and use of Lepidopterous pest insects and their natural enemies as biocontrol agents.

(2) Develop a fundamental understanding of environmental, physiological and genetic regulation of diapause in Lepidopterous insects.

(3) Use knowledge gained from (1) and (2) above to support, improve or develop alternative and environmentally acceptable strategies for insect pest control.

7. **Research priorities in your program:**

(1) Determine the role of environmental factors in regulating expression of the diapause response.

(2) Determine the genetic factors that regulate diapause.

(3) Determine the nature of the Neuro-endocrine mechanisms which mediate environmental control of insect diapause or development (emphasis on photo-neuroendocrine system).

(4) Develop methods for manipulation of diapause in the laboratory (to permit year-round production and stockpiling of pestiferous and beneficial insects for biocontrol programs).

(5) Based on information from (1) and (2), develop a conceptual and computer model to predict the time and duration of diapause in different climatic zones in North America (special emphasis on the gypsy moth).

8. **Progress of current research in solving problems:**

The only environmental factor that seems to regulate the diapause response of the gypsy moth is temperature which modifies intensity of the response. A nearly homozygous, nondiapause genotype (NDP) has been developed by continuous selection. Reciprocal crosses of NDP and wild (DP) genotypes indicate polygenic control with a strong maternal influence. The maternal influence may involve a diapause hormone which is secreted by the female into developing oocytes as is known to occur in Asian silkworm, Bombyx mori.

9. **Significant research accomplishments in the past 3 years:**

(1) Development and refinement of technology for improved laboratory rearing and large scale production of the gypsy moth.

(2) Development of non-diapausing and short-diapausing strains of the gypsy moth.

(3) Discovered, colonized and determined genetics of an albino mutant strain with Pleiotropic expressions.

(4) Determined that diapause intensity in gypsy moths differs depending upon temperature, sex and number of female larval instars.

(5) Contributed to development of monoclonal antibodies for PTTH (University of Maryland) and ultrastructural evidence of neurosecretory cells believed to produce diapause hormone in the gypsy moth (University of Massachusetts).

10. Impact of research accomplishments on science and the general public:

Development of mass rearing technology has led directly to more efficient large scale production of pathogens, parasites and sterile males. Operational use of such biological and autocidal control strategies can result in decreased reliance on conventional broad spectrum pesticides.

Research relating to ecology, physiology and genetics of insect diapause not only supports efficient mass production of insects and their natural enemies but also provides strains of insects with short generation times to greatly increase the rate at which experiments can be carried out. Also, new pesticides may be developed based on neuropeptide analogs that can selectively inhibit development or precociously terminate diapause in insects.

11. Obstacles to achieving objectives:

(1) Lack of knowledge relating to neurohormonal mechanisms involved in the induction, maintenance and termination of diapause.

(2) Lack of expertise working in a concerted team effort to solve the problem.

(3) Lack of suitable bioassay for PTTH and diapause hormone such as specific monoclonal antibodies to probe the role of these neurohormones in regulation of development.

(4) Location of research activity is inappropriate for interaction of expertise required to solve the problem.

12. Future lines of needed research and plan for implementation:

(1) Determine the genetic factors involved in the regulation of diapause (frequency distribution analysis of diapause response of progeny (F_1 and F_2) from crosses and backcrosses of non-diapausing and diapausing genotypes, to be completed by 1986).

(2) Determine the role of environmental factors involved in expression of the diapause response (the intensity of the diapause response will be determined for insects whose developmental stages are exposed to various temperature and humidity conditions).

(3) Neuroendocrine regulation of diapause will be determined by using the non-diapause and diapause strain in comparative, ultrastructural, histophysiological including endocrine and neuroendocrine assays. The hypothesis will be tested that a diapause hormone is involved.

13. Research facilities and personnel needs:

The present location is unfavorable for carrying out research objectives. Key support personnel, and scientific expertise is not available. It is recommended that the research should be carried out at the Beltsville Agricultural Research Center in cooperation with scientists trained in genetics, biochemistry, neuroendocrinology and physiology. Additionally, instrumentation is also available there.

14. Extent of cooperation--names of persons and institutions:

Dr. Michael Ma - Department of Entomology
University of Maryland

Dr. Chi Yin - Department of Entomology
University of Massachusetts

Dr. J. Svoboda - Insect Physiology Laboratory

Dr. D. Hayes - Livestock Investigations Laboratory
Beltsville

15. Titles of publications for the last 3 years: (not particularly applicable to peptide workshop??)

Bell, R.A., C.D. Owens, M. Shapiro and J.R. Tardif (1981) Development of Mass Rearing Technology in: The Gypsy Moth: Research Toward Integrated Pest Management, pp 599-633, USDA Tech Bull. 1584

Bell, R.A. (1984) Role of the Frontal Ganglion in Lepidopterous Insects in: Insect Neurochemistry and Neurophysiology (Ed: Borkovec, A.B. and T.J. Kelly) pp. 321-323. Plenum Press N.Y.

T.M. ODell, R.A. Bell, V.C. Mastro, J.A. Tanner and L.F. Kennedy (1984) Production of the Gypsy Moth, Lymantria Dispar for Research and Biological Control (In: King, E.C. and N.C. Leppla Ed: Advances and Challenges in Insect Rearing) pp. 156-166, USDA - ARS (Southern Region)

Raina, A.K., R.A. Bell and W. Klassen (1981) Diapause in the Pink Bollworm: Preliminary Genetic Analysis Insect Sci. Application 1(3): 231-235

1. Scientist's name, address, and telephone number:

Dr. Douglas M. Moore, Ph.D. COM: 516-323-2500
Acting Laboratory Chief, Molecular Biology FTS: 349-9309
PIADC, NAA, USDA
P.O. Box 848
Greenport, NY 11944

2. Location:
Plum Island Animal Disease Center
Section - Molecular Biology Laboratory

3. Number and title of CRIS work unit:

1940-20460-093 (pending, CRIS replacement for 1502-20460-018)
Amino acid sequences in foot-and mouth disease virus types and immunogenic peptide fragments.
Also, 1940-20460-091A Synthetic Peptides, Brookhaven National Lab., (Cooperator)

4. Approach Element and Problem Definitions:

3.4.1 Improve methods for diagnosing and identifying agents that cause losses.
3.4.3 Characterize the mechanism by which animals become infected or are affected by diseases.
3.4.4 Devise new and improved methods for preventing or reducing death, morbidity or other losses from animal diseases.

5. Estimated SY's:

Inhouse 1.4 SY - Extramural 1.0 SY

6. Objectives of research:

To indentify polypeptide amino acid sequences from the viral genome coding sequence which play a role in the immunogenic properties of foot-and-mouth disease virus (FMDV). Chemically synthesize peptide antigens to assess the relevance of these sequences to native antigenic structures in the virus particle and on the functional virus encoded proteins. Prepare polypeptide antigens by subfraction of virus proteins and compare these to synthetically produced peptide antigens. Determine the activity of these protein and peptide antigens when used as experimental vaccines or as reagents for the detection and/or quantitation.

6a. Technical Capabilities and available facilities::

Expertise - Gene cloning, DNA sequencing and related analytical techniques.
In vitro protein translation, protein post-transcriptional processing.
Protein and peptide purification.
Protein sequence analysis, amino acid analysis.
Peptide solid phase synthesis (manual "hand" operation).

7. Research priorities in your program:

The research priorities in our research program are to study the means by which livestock become immune to foreign animal diseases and to devise methods to immunize against infection (especially for Foot-and-mouth disease). This involves exploring new methods of vaccine development which include cloned protein expression of vaccine antigens and the study of synthetic peptides as antigens.

8. Progress of current research in solving problems:

Work at Plum Island has demonstrated the principle of a cloned protein vaccine for FMDV. This was possible through basic studies on FMDV proteins identifying effective virus antigens on isolated proteins of the virus. Subsequent work in collaboration with Genentech, Inc., demonstrated the first effective protein vaccine for a disease of livestock or man. Current work is directed toward identifying the chemical basis for antigenic variation of FMDV and the discovery of effective antigens which can be produced synthetically by organic synthesis.

9. Significant research accomplishments in the past 3 years:

First demonstration of an effective protein vaccine produced by recombinant DNA methods for a disease of animals or man.

Identification of the complete amino acid sequence of the FMDV RNA polymerase enzyme and the genome location of the gene sequence for this viral enzyme.

Identification of the VP-1 protein exposed tyrosine amino acid identifying the exposed region of the immunogenic protein on the surface of the viral capsid.

10. Impact of research accomplishments on science and the general public:

The impact of the research on science has been to dempnstrate the feasibility of producing a protein (or "subunit") vaccine using an isolated viral protein.

The demonstration of a cloned viral protein vaccine produced by recombinant DNA methods has had a major impact on science and been the first of now a growing number of succesful experiments to find alternate sources of antigens for viral vaccines

The impact on the general public is the significant progress toward the availability of a new vaccine for Foot-and-mouth disease. It is expected that this new vaccine will be available by the end of this decade or possibly sooner. The effect of this new vaccine on animal disease control in the third world may be quite significant in the control and eradication of FMD.

11. Obstacles to achieving objectives:

The main obsticle to achieving objectives for this work is the lack of knowledge of the complex arrangement of the viral proteins in the FMDV capsid. This work on the physical structure of the virus capsid is just beginning in the area of picornavirus studies and will not be available for some years to come. Therefore, present studies are directed to the chemical characterization of the numerous antigenic sites found on the virus capsid. Thus, an empirical structure of the antigens of the virus are being made. This coupled with data obtained by other chemical studies will enable a definition of the antigen "structure" of the virus and allow development of additional viral antigens through cloning or synthetic sources.

12. Future lines of needed research and plan for implementation:

The plan for future needed research is to characterize the numerous antigens of the virus through the use of monoclonal antibodies and synthetic peptide studies. This data will provide the basis for defining important antigenic sites of the virus for further development. As structural and chemical data are obtained for the structure of the virus, the results will be integrated to define the more complex antigenic sites found on the surface of the virus particle. It may be possible to define and synthesize the highly complex antigens which exist only on the intact infectious virus and thus produce better vaccine antigens biosynthetically or by organic synthesis.

13. Research facilities and personnel needs:

The needs for this research are generally being met with existing facilities, staff and cooperators. However, the need for supply of synthetic peptides far exceeds the present supply which is obtained through hand synthesis methods. It is anticipated that obtaining an automated solid phase peptide synthesis machine will supply the needed peptides.

14. Extent of cooperation--names of persons and institutions:

Peptides: Soliman Bakr - UDSA cooperative agreement with the Dept. of Energy
Biology Department
Brookhaven National Laboratory
Upton, New York

Cloned viral proteins:
Dr. Dennis G. Kleid
Department of vaccine Development
Genentech, Inc.
460 Point San Bruno Blvd.
South San Francisco, CA

15. Titles of publications for the last 3 years:

Kleid, D.G., et al. 1981. Cloned viral protein vaccine for Foot-and-mouth disease: responses in cattle and swine. Science 214: 1125-1129.

Robertson, B.H., et al. 1983. Identification of an exposed region of the immunogenic capsid polypeptide VP1 on foot-and-mouth disease virus. J. Virology 46:311-316.

Robertson, B.H., et al. 1983. Identification of amino acid and nucleotide sequence of the foot-and-mouth disease virus RNA polymerase. Virology 126: 614-623.

Moore, D.M. 1983. Production of a vaccine for foot-and-mouth disease through gene cloning. In: Beltsville Symposium VII, Genetic Engineering: Applications to Agriculture, pp 89-106. Ed. L.D.Owens, Rowman and Allanheld, Totowa.

Grubman, M.J. 1984. A biochemical map of the polypeptides specified by foot-and-mouth disease virus. J. Virology 50:579-586.

1. Scientist's name, address, and telephone number:

T. S. Adams
Met Lab USDA
State University Station
Fargo, ND 58105

COM: 701-237-5771
FTS: 783-5471

2. Location:

Metabolism and Radiation Research Laboratory
P.O. Box 5674, State University Station
Fargo, ND 58105

3. Number and title of CRIS work unit:

315-20252-002
Develop New and Improved Principles and Practices of Arthropod Control based on the Selective disruption of their Growth, Development and Reproduction

4. Approach Element and Problem Definitions:

3.19 Develop fundamental understanding of insect control technology
3.19.2 Biochemical regulation
Project Area-Develop a fundamental understanding of biochemistry and physiology of reproduction

5. Estimated SY's:

1 SY

6. Objectives of research:

(1) Problem.-Reproduction in insects is influenced and regulated by numerous cnemical and physical factors. Only in the last few decades first inroads were made into the understanding of its biochemical regulation and it is now generally agreed that the development and function of reproductive organs is controlled, at different levels, by a considerable variety of endogenous biochemical agents. Although a small number of these agents, e.g., the juvenile and molting hormones, were already characterized, the full scope and mechanism of their action is still not clearly understood. Anotner group of neural origin, constitutes the brain hormones, neurohormones and related factors with tentatively assigned roles by unknown structures (Riddiford, 1980. Insect endocrinology. Ann. Rev. Physiol. 42:511-28.) The isolation and characterization of these compounds, the determination of their mode of action, and the elucidation of mechanisms by which insect reproduction is regulated represents an important segment of invertebrate biology open to study and clarification.

7. Research priorities in your program:

a.) Determine the role of JH, Ecdysteroids, EDNH and the oostatic hormone in dipteran reproduction (pheromone biosynthesis, mating behavior, oogenesis, vitellogenesis).

b.) Show hormone interaction and feedback patterns.

c.) Develop a specific bioassay for the oostatic hormone.

d.) Isolate and identify methylalkanes with sex pheromone activity.

8. Progress of current research in solving problems:

Have shown that JH, EDNH, and Ecdysteroids are all involved in pheromone production, vitellogenin synthesis and oogenesis.

Have developed in vitro systems for vitellogenin synthesis, oogenesis, and pheromone synthesis. Thus can study individual hormones action on specific tissues.

9. Significant research accomplishments in the past 3 years:

1.) Have shown that 20-OH-ecdysone turns on biosynthesis of muscalure (Z-(9)-Tricosene) by altering the pattern of cuticular hydrocarbon biosynthesis. Males never make muscalure but an injection of 20-OH-ecdysone will induce its biosynthesis.

2.) Vitellogenin synthesis is also stimulated by 20-OH-ecdysone. Males will also make vitellogenin after the injection of 20-OH-ecdysone.

3.) Have shown the roles of each component of the housefly sex pheromone in mating behavior.

10. Impact of research accomplishments on science and the general public:

My work on the oostatic hormone in Musca has stimulated others to conduct research in this area. See "OOstatic Hormone"-Antigonadotropin and Reproduction" pp. 319-329 by Erwin Huebner in Endocrinology of Insects ed. R. G. H. Downer and H. Laufer, Alan R. Liss Inc. c.1983.

As a result of my research in Insect reproduction, 2 book chapters were written from invitational papers given at international symposia.

Endocrine Control of Pheromone Biosynthesis and Mating Behavior in the Housefly, Musca domestica pp. 441-457. Advances in Invertebrate Reproduction 3 ed. Engels, Clark, Fischer, Olive, Went c.1984.

The role of Ovarian Hormones in Maintaining Cyclical Egg Production in Insects pp. 109-125. Advances in Invertebrate Reproduction ed. Clark and Adams c.1981

11. Obstacles to achieving objectives:

Could use the assistance of an electrophysiologist to assist in the development of a specific bioassay for the oostatic hormone based on electrical changes in neurohaemal storage areas during hormone release. All assays for the oostatic hormone in diptera, to date, are non-specific and are based on the retardation of egg development.

12. Future lines of needed research and plan for implementation:

Develop electrophysiological assay for the oostatic hormone. Will attempt to establish a cooperative research program with Dr. Jeffery Gerst an electrophysiologist in the Zoology Dept. at NDSU. We will write up a competetive grant for this work.

13. Research facilities and personnel needs:

a. Could use an electrophysiologist on a 2-year postdoctoral appointment.

b. Capillary G.C. MS and purse trap system to isolate hydrocarbon fractions.

14. Extent of cooperation--names of persons and institutions:

Henry Hagedorn	Cornell University
Gary Blomquist	University of Nevada, Reno
Pete Massler	Insect Reproduction Lab., USDA Beltsville, MD
Tom Kelly	Insect Reproduction Lab., USDA Beltsville, MD
Howard Jaffey	Vet. Tox. USDA Beltsville, MD
Dennis Nelson	Met. Lab. USDA Fargo, ND

15. Titles of publications for the last 3 years:

Haemolymph Ecdysteroid in the housefly Musca domestica during oogenesis and its relationship with vitellogenen levels. J. Insect Physiology.

Endocrine control of pheromone biosynthesis and mating behavior in the housefly, Musca domestica. Adv. Inv. Rep.

Induction of female sex pheromone production in male houseflies by ovary implants or 20-OH-ecdysone.

The role of 20-OH-ecdysone in housefly sex pheromone biosynthesis.

Vitellin and vitellogenin concentrations during oogenesis in the first gonotrophic cycle of the housefly, Musca domestica.

Regulation of oogenesis, vitellogenin levels and mating in the housefly Musca domestica and its disruption by natural products or their analogues. Proc. Int. Conf. Nat. Prod. as Reg. Insect Repr. Jammu (in press)

Correlation of housefly sex pheromone production with ovarian development. J. Insect Physiology.

Morphology of the male reproductive tract of mature, larval, pupal, and adult tobacco hornworms, Manduca sexta. Ann. Ent. Soc.

1. Scientist's name, address, and telephone number:

Edwin P. Marks
3608 Fairway Road 701-232-4562
Genetics and Cell Biology Section 701-237-5771- ext. 451

2. Location:

Metabolism and Radiation Research Laboratory
P.O. Box 5674, State University Station
Fargo, ND 58105

3. Number and title of CRIS work unit:

3915-20250-001

4. Approach Element and Problem Definitions:

2.4.01.1.b Metabolism and secretion of components of the insect cuticle.

2.4.01.2.a Hormone action, secretion, and metabolism.

5. Estimated SY's:

1

6. Objectives of research:

(1) To investigate the mechanisms which control chitin synthesis in insects and devise ways to interrupt this process.

(2) To determine the role of hormones in the control of cuticle formation.

(3) To determine the mode of action of chitin synthesis inhibitors.

(4) To develop cell culture bioassays for bioactive compounds.

7. Research priorities in your program:

(1) Development and application of quantitative cell culture bioassays for chitin synthesis.

(2) Development of automatable bioassay for chitin synthesis inhibitors.

(3) Investigate how chitin is secreted and integrated into the developing cuticle.

(4) Determine the role of hormones in the control of chitin synthesis and secretion.

8. Progress of current research in solving problems:

(1) Have demonstrated that hormones alter properties of cell surface membranes.

(2) Have screened 11 cell lines for chitin synthesis and selected three cell lines for further investigation.

(3) Have shown that exogenous 20-hydroxyecdysone is not required for chitin synthesis in cell lines but may accelerate it in some lines.

(4) Have shown that diflubenzoron inhibits chitin synthesis with variable results in different cell lines.

9. Significant research accomplishments in the past 3 years:

(1) Developed cell culture bioassay for chitin synthesis and chitin synthesis inhibitors that has the potential for automation.

(2) Developed new hypothesis for the mode of action of chitin synthesis inhibitors.

10. Impact of research accomplishments on science and the general public:

Two manufacturers of agricultural chemicals, Ciba Geigy and Monsanto have expressed interest in using this new bioassay for chitin synthesis inhibitors.

Two foreign researchers, Delachambre and Mauchamp have inquired about possible collaborative efforts.

11. Obstacles to achieving objectives:

(a) Personnel to run experiments is the greatest need and
(b) Time on the part of the senior scientist.

When the myogenic peptides studied by Holman and Cook are available in ^{14}C labelled form we will continue studies on the biosynthesis and uptake of neuropeptides by cultured insect brains that was halted some years ago by the need for such labelled peptides.

12. Future lines of needed research and plan for implementation:

The availability of labelled neuropeptides in quantity will open the way to studies on receptor mechanisms, mode of action, and biosynthetic pathways that will be most effectively carried out in cell cultures. Bioassays at the cellular level are urgently needed. They will involve the screening of existing insect cell lines for response to the new peptides and for ability to synthesize and release these compounds.

13. Research facilities and personnel needs:

The research facility at MRRL was set up 20 years ago on a temporary basis and is still being used. The greatest need is for a GS 7 or 9 technician to maintain the cell lines and operate the facility so that the scientist can concentrate on research and writing.

14. Extent of cooperation--names of persons and institutions:

Cooperative effort at the present time is being carried out with Dr. Judith Willis, University of Illinois, Urbana. Work involves production of cuticular proteins and chitin in cultured tissues and cells.

15. Titles of publications for the last 3 years:

Marks, E. P., T. Leighton, and F. Leighton. Modes of action of chitin synthesis inhibitors. In Coats, J. (ed.) Insecticide Modes of Action, Academic Press, NY. Part III, Chapt. 10, pp. 277-309, 1982.

Marks, E. P. Insect tissue culture systems: Tools for insect endocrinology. In Laufer, H. and R. Downer (eds.) Insect Endocrinology. Allan R. Liss Inc., NY. Chapter 2, pp. 509-515, 1983.

Jang, E. B., and E. P. Marks. The effects of tunicamycin and 20-hydroxyecdysone on the cell surface properties of two insect cell lines. Arch. Ins. Biochem. Physiol. 1:59-71, 1983.

English, L. H., B. Magelky, and E. P. Marks. 20-hydroxyecdysone-induced changes in lepidopteran cell line-MRRL-CH associated with popular dynamics. In Vitro. 20:71-78, 1984.

Marks, E. P., E. B. Jang, and R. Stolee. Incorporation and metabolism of N-acetylglucosamine by a lepidopteran cell line. In Kurstak, Maramorosch and Oberlander (eds.) Invertebrate Systems In Vitro. Elsevier/North, Holland, NY. (Book Chapter) 1984 (in press)

Jang, E. B., H. Klosterman, and E. P. Marks. Effects of 20-hydroxyecdysone and tunicamycin on glycoprotein synthesis in an insect cell line. Arch. Insect Biochem. Physiol. 1:251-266, 1984.

Marks, E. P., J. Balke, and H. Klosterman. Evidence for chitin synthesis in an insect cell line. Arch. Insect Biochem. Physiol. 1:225-230, 1984.

Marks, E. P. The Control of Chitin Synthesis: Mechanisms and Methods. In Wright, J. (ed.) Chitin and the Benzyolphenylureas. Junc Inc. Pub. (Book Chapter) 1985 (accepted for publication)

Leopold, R. A., E. P. Marks, J. Eaton, and J. Knoper. Ecdysial failures in the cotton boll weevil: Synergism of diflubenzuron with juvenile hormone. Submitted to Pesticide Biochem. Physiol.

1. **Scientist's name, address, and telephone number:**

Andrew C. Chen, Actin Research Leader
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P.O. Drawer GE
College Station, TX 77841
COMM: 409-260-9401 FTS: 527-1401

2. **Location:**

College Station, Texas

3. **Number and title of CRIS work unit:**

6202-20482-001 - Physiological and toxicological studies of those arthropods that affect livestock

4. **Approach Element and Problem Definitions:**

3-5-03-1a PRODUCTIVITY/QUALITY--ANIMALS
CONTROL OF INSECTS, TICKS, & MITES
REDUCE LOSSES-ANIMALS & MAN

5. **Estimated SY's:** 6 - One not working on neuropeptides

6. **Objectives of research:**

Identify and characterize hormones, neurohormones, enzymes, proteins, and other chemicals produced by arthropods that affect livestock and determine their role and effects, as well as the effects of toxicants, sterilants, and other chemicals on insect morphology, physiology and biochemistry.
Special technical capabilities: (1) *In vitro* translation of RNA
(2) Intralymph node immunization of rabbits.

7. **Research priorities in your program:**

(1) Metabolism of livestock insects and neuroendocrine modulation of metabolism. These metabolic processes include amino acid metabolism, carbohydrate metabolism and excretion.
(2) Endocrine control of development, specifically ovarian development.

8. **Progress of current research in solving problems:**

(1) Purified 2 enzymes involved in chitin metabolism.
(2) Purified trehalase that hydrolyzes trehalose and is critical in the study of of carbohydrate metabolism.
(3) Identified and purified a yolk protein and produced antiserum against it.

9. **Significant research accomplishments in the past 3 years:**

(1) Developed a fluorescent assay for the quantitation of amino sugars.
(2) Disproved that the chitin synthesis inhibitors inhibit chitin synthesis direct
(3) Determined the hemolymph constituents of the adult stable fly. This serves as a foundation for the study of diuresis and its endocrine regulation.

10. Impact of research accomplishments on science and the general public:

The discovery of non-inhibition of chitin synthase by "chitin synthesis" inhibitors has caused researchers to look for alternate mode of action of these compounds.

11. Obstacles to achieving objectives:

Insufficient funding and lack of technical assistance.

12. Future lines of needed research and plan for implementation:

Many metabolic pathways that may be regulated by endocrine systems have not been explored. The may be exploited for insect control strategies.

13. **Research facilities and personnel needs:**

A. FACILITIES:

1. Microcomputer
2. Refrigerated microcentrifuge
3. Two dimensional scanning densitometer
4. Flow-through radioactivity monitor

B. PERSONNEL:

1. One full-time technician.
2. Post-doctoral research associate

14. **Extent of cooperation--names of persons and institutions:**

None currently

15. **Titles of publications for the last 3 years:**

SEE ATTACHED

1. Chen, A. C. Neuroendocrine modulation of carbohydrate metabolism. Southwest. Entomol. Suppl. 5:17-23. 1983.

2. Mayer, R. T., Chen, A. C. and DeLoach, J. R. Characterization of mannosyl transferases during the pupal instar of Stomoxys calcitrans (L.). Arch. Insect Biochem. Physiol. 1(1):1-15. 1983.

3. Mayer, R. T. and Chen, A. C. Effects of diflubenzuron and tunicamycin on N-acetylglucosaminyl transferases in prepupae of the stable fly (Stomoxys calcitrans). Experientia In press.

4. Mayer, R. T. and Chen, A. C. N-Acetylglucosaminyl transferases from the pupal instar of the stable fly, Stomoxys calcitrans. Arch. Insect Biochem. Physiol. In press.

5. Chen, A. C. and Mayer, R. T. Insecticides: Effects on the Cuticle. In Kerkut, G. A. and Gilbert, L. I. (eds) Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press, New York. In press. (Book Chapter)

1. Scientist's name, address, and telephone number:

Benjamin J. Cook COMM: 409-260-9401
USDA, ARS FTS: 527-1401
Veterinary Toxicology & Entomology Laboratory
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2. Location:

College Station, Texas

3. Number and title of CRIS work unit:
6202-20482-001
Physiological and Toxicological Studies of Those Arthropods that Affect Livestock

4. Approach Element and Problem Definitions:

3.5.03.1a Productivity/Quality--Animals Control of Insects, Ticks, and Mites Reduce Losses-Animals and Man
Lack of basic knowledge of the biochemistry and physiology of insects, ticks, and mites that affect animals and humans prevents pursuit of new concepts for developing control methods for those pests.

5. Estimated SY's:
6 for the unit.
(one of these is not involved in neuropeptides)

6. Objectives of research: As stated in the current CRIS Unit.

Identify and characterize hormones, neurohormones, enzymes, proteins and other chemicals produced by arthropods which affect livestock and determine their role and effects, as well as the effects of toxicants, sterilants, and other chemicals on insect morphology, physiology, and biochemistry.

7. Research priorities in your program:

1. Determine mode of action of isolated myotropic peptides on various physiological systems.

2. Develop RIA's to (a) Determine tissue distribution in the cockroach and stable fly.

3. Study the neuroendocrine complex in the dorsal diaphragm of the stable fly.

8. Progress of current research in solving problems:
Excellent.

1. Discovered evidence for 2 Ca transmembrane channels in the regulation of insect visceral muscle.

2. Found large amount of the Ca regulating protein Calmodulin in the viscera of insects.

9. Significant research accomplishments in the past 3 years:

1. Measured physiological amino acids and related compounds in the brain and thoracic ganglion of the stable fly.

2. Isolated and partially characterized a second myotropic peptide from the hindgut of the cockroach.

3. Studied the role of proctolin and glutamate in the excitation-contraction coupling of insect visceral muscle.

4. Determined the distribution of Calmodulin in the tissues of the cockroach and several other insect species.

5. Described heart structure and beat in the stable fly.

10. Impact of research accomplishments on science and the general public:

On science - large

Two insect peptides have been known for almost 9 years, proctolin and AKH. Only in September 1984 were two cardiotropins added to this list. Our discovery of 15-20 cephalo myotropins will greatly expand the availability of insect peptides to the scientific community for a wide variety of biochemical and physiological studies.

11. Obstacles to achieving objectives:

Lack of funding.
Lack of technical support--more technicians.
Some new instruments needed.

12. Future lines of needed research and plan for implementation:

1. Determine other physiological and biochemical function for myotropic peptides. Develop new bioassays to evaluate the peptides and their analogs.

2. Explore 2nd messenger mechanisms in insect viscera.

13. Research facilities and personnel needs:

Current equipment available:
Cambridge S-4 SEM
Waters ALC-100 HPLC
Waters 201 HPLC
Applied Biosystems 470A Protein Sequencer
Waters-LDC HPLC
VG Mass Spec. 70-250
Amplifiers, Stimulators, O-Scope, Recorders

14. Extent of cooperation--names of persons and institutions:

There is an insect neuropeptide group at Texas A&M University. This group, headed by Dr. L. L. Keeley is composed of 16 individuals (scientists, post-doctorals, graduate students, and technicians). They are also in the process of updating and adding new equipment. Like our unit, they will certainly become a leading group in the discovery of insect neuropeptides. Although we have no joint ventures with TAMU at the present time, preliminary discussions about cooperation have been most promising.

Dr. R. Meola, Texas A&M University.

15. Titles of publications for the last 3 years:

Cook, B. J. Insect Myotropic and Neurotropic Factors. In Downer, R. G. H. and Laufer, H. (eds.) Endocrinology of insects, Alan R. Liss, New York. pp. 467-484. 1983. (Book Chapter).

Cook, B. J. and Meola, S. Heart structure and beat in the stable fly, Stomoxys Calcitrans. Physiol. Entomol. 8:139-149. 1983.

Cook, B. J. Insect neuropeptides - An overview. Southwest. Entomol. Suppl. 5:2-8. 1983.

Cook, B. J. and Holman, G. M. Peptides and Kinins. In Kerkut, G. A. and Gilbert, L. I. (eds.) Comprehensive Insect Physiology, Biochemistry and Pharmacelogy, Pergamon Press, New York. 1984. (Book Chapter) (in press)

Cook, B. J., Holman, G. M., and Meola, S. The oviduct musculature of the cockroach, Leucophaea maderae and its response to various neurotransmitters and hormones. Arch. Insect Biochem. Physiol. 1(2):167-178. 1984.

Cook, B. J. and Holman, G. M. The role of proctolin and glutamate in the excitation-contraction coupling of insect visceral muscle. Comp. Biochem. Physiol. 1984. (in press).

Holman, G. M. and Cook, B. J. Physiological amino acids of the nervous system of the stable fly, Stomoxys calcitrans. Comp. Biochem. Physiol. 71A:23-27. 1982.

Holman, G. M. and Cook, B. J. Isolation and partial characterization of a second myotropic peptide from the hindgut of the cockroach, Leucophaea maderae. Comp. Biochem. Physiol. 76C:39-43. 1983.

Holman, G. M. and Cook, B. J. Do insect have tachykinins? Southwest. Entomol. Suppl. 5:24-25. 1983.

Holman, G. M., Cook, B. J., and Wagner, R. M. Isolation and partial characterization of five myotropic peptides present in head extracts of the cockroach, Leucophaea maderae. Comp. Biochem. Physiol. 77C:1-5. 1984.

Holman, G. M., Cook, B. J. Proctolin, its presence in and action on the oviduct of an insect. Comp. Biochem. Physiol. 1984. (in press).

Wright, M. and Cook, B. J. Studies on the mode of action of proctolin. Southwest. Entomol. Suppl. 5:26-32. 1983.

Wright, M. S. and Cook, B. J. Distribution of Calmodulin in insects as determined by radioimmunoassay. Comp. Biochem. Physiol. 1984. (in press).

1. Scientist's name, address, and telephone number:

G. Mark Holman
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COMM: 409-260-9322
FTS: 527-1322

2. Location:

Phys. & Biochem. of Livestock Ins. Research Group

3. Number and title of CRIS work unit:
7302-20482-001
Physiological and Toxicological Studies of Those Arthropods that Affect Livestock

4. Approach Element and Problem Definitions:

3.5.03.1a Productivity/Quality--Animals Control of Insects, Ticks, and Mites Reduce Losses-Animals and Man
Lack of basic knowledge of the biochemistry and physiology of insects, ticks, and mites that affect animals and man.

5. Estimated SY's:
6 for the unit.
(one of these is not involved in neuropeptides)

6. Objectives of research: From the CRIS Unit write-up verbatum.

Identify and characterize hormones, neurohormones, enzymes, proteins and other chemicals produced by arthropods which affect livestock and determine their role and effects, as well as the effects of toxicants, sterilants, and other chemicals on insect morphology, physiology, and biochemistry.

7. Research priorities in your program:

1. Isolate and identify myotropic peptides

2. Determine other functions of these peptides. i.e. Diuretic and antidiuretic hormones, hyper and hypotrehalosemic hormones, etc.

3. Evaluate analogs

4. Develop RIA's to (a) Determine distribution in the roach and
(b) Presence in the stable fly.

5. Study post-translational modifications.

8. Progress of current research in solving problems:

Excellent - 16 peptides isolated in pure form and on hand
4 molecular weights known
2 partial sequences

Hope to have sequences of 18-20 new peptides within the next 6 months if our new equipment operates anywhere near the spec's.

9. Significant research accomplishments in the past 3 years:

(a) Measured physiological amino acids and related compounds in the brain and thoracic ganglion of the stable fly.

(b) Discovered and partialy purified and characterized a second myotropic peptide in the cockroach hindgut. The peptide is also present in Malpigian tubule extracts.

(c) Demonstrated several peptides present in roach head extracts.

(d) Demonstrated and quantified the presence of proctolin in extracts of the cockroach oviduct. First time proctolin has been shown to be present in the reproductive system.

(e) Isolated 16 myotropic peptides from cockroach head extracts. Purified these to single, pure compounds. Characterized each with enzymes. Have molecular weights on 4 and partial sequences on two.

10. Impact of research accomplishments on science and the general public:

On science - Large.

Only 4 insect neuropep. structures known, proctolin for 8 years and the most recent two only a few months. Proctolin is generating about 1 publication/month at the present time. 18-20 new peptides available for study to the science community at large could generate more than 100 papers per year for several years.

11. Obstacles to achieving objectives:

Lack of Funding.
Lack of Technical Support (need technicians).
Some new instrumentation needed.

12. Future lines of needed research and plan for implementation:

1. Evaluation of peptide analogs of the peptides we identify.

2. Definition of other biochemical and physiological functions of our peptides other than stimulation of visceral muscle.

3. Peptide biosynthesis - Post translational modifications, etc.

1. Develop new bioassays to evaluate effects of our peptides on other systems. Evaluate analogs in those systems.

13. Research facilities and personnel needs:

(1) Need to replace an 11 year old HPLC that has been the backbone of my effort.

(2) Need hands.

(3) Unit needs a Cat. 3 scientist to run and maintain some of the sophisticated instruments that have a service component.

14. Extent of cooperation--names of persons and institutions:

Roger Meola - TAMU

Hope to have coop. project with Keeley & Hayes at TAMU in near future. At present stage we simply consult on research problems that are common to both neuropeptide groups.

15. Titles of publications for the last 3 years:

Holman, G. M. and Cook, B. J. Physiological amino acids of the nervous system of the stable fly, Stomoxys calcitrans. Comp. Biochem. Physiol. 71A:23-27. 1982.

Spates, G. E., Stipanovic, R. D., Williams, H. and Holman, G. M. Mechanism of haemolysis in a blood-sucking dipteran, Stomoxys calcitrans. Insect Biochem. 12(6):707-712. 1982.

DeLoach, J. R., Spates, G. E. and Holman, G. M. Analysis of a defined diet for adult Stomoxys calcitrans (Diptera: Muscidae). Radiation techniques and their application to insect pests. I.A.E.A. Circ. No. 30. 1982. (Abstract)

Holman, G. M. and Cook, B. J. Do insects have tachykinins? Southwest Entomol. Suppl. 5:24-25. 1983.

Sylvia, V. L. and Holman, G. M. Isolation and high-performance liquid chromatographic purification of a myotropic peptide from the hindgut of the crayfish, Procambarus clarkii. J. Chromatogr. 261:158-162. 1983.

DeLoach, J. R. and Holman, G. M. An amino acid analysis of freeze-dried bovine and porcine blood used for in vitro rearing of Glossina palpalis palpalis (Rob.-Desv.). Comp. Biochem. Physiol. 75A:499-502. 1983.

Holman, G. M. and Cook, B. J. Isolation and partial characterization of a second myotropic peptide from the hindgut of the cockroach, Leucophaea maderae. Comp. Biochem. Physiol. 76C:39-43. 1983.

Cook, B. J. and Holman, G. M. Peptides and Kinins. In Kerkut, G. A. and Gilbert, L. I. (eds.) Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Pergamon Press, New York. (Accepted by editors on May 2, 1983) (Book Chapter).

Holman, G. M., Cook, B. J. and Wagner, R. M. Isolation and partial characterization of five myotropic peptides present in head extracts of the cockroach, Leucophaea maderae. Comp. Biochem. Physiol. 77C:1-5. 1984.

Cook, B. J. and Holman, G. M. Proctolin and second myotropic peptide from the hindgut of the cockroach, Leucophaea maderae. Proc. Int. Conf. Insect Neurochem. Neurophysiol. 345-348. Plenum 1984.

Holman, G. M., Cook, B. J. and Wagner, R. M. Isolation of myotropic factors from the head of the cockroach, Leucophaea maderae. Proc. Int. Conf. Insect Neurochem. Neurophysiol. 381-384. Plenum 1984.

Wagner, R. M. and Holman, G. M. Microsequencing strategies for insect neuropeptides. Proc. Int. Conf. Insect Neurochem. Neurophysiol. pp. 251-264. Plenum 1984.

Cook, B. J., Holman, G. M. and Meola, S. M. The oviduct musculature of the cockroach, Leucophaea maderae and its response to various biogenic amines and hormones. Arch. Insect Biochem. Physiol. 1(2):167-178. 1983.

Cook, B. J. and Holman, G. M. The role of proctolin and glutamate in the excitation-contraction coupling of insect visceral muscle. Comp. Biochem. Physiol. 1984 (in press).

Holman, G. M. and Cook, B. J. Proctolin, its presence in and action on the oviduct of an insect. Comp. Biochem. Physiol. 1984 (in press).

Wright, M. S., Cook, B. J. and Holman, G. M. Purification of calmodulin from insect visceral muscle by high-performance liquid chromatography. Fed. Proc. 43: 1579. May 1984

1. Scientists name, address and telephone number

Shirlee Meola FTS 527-1339
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PO Box GE
College Station, TX

2. Location:
Veterinary Toxicology and Entomology Research Laboratory
Physiology and Biochemistry of Livestock Insect Unit

3. Number and title of CRIS work unit

CRIS 7302-20482-001
Physiological & toxicological studies of those arthropods affecting livestock

4. Approach Element and Problem Definitions:
3.5.03.1a Productivity/Quality ---- Animals
Control of Insects, Ticks, & Mites
Aquisition of basic knowledge of morphology, biochemistry and physiology of insects, ticks and mites that affect livestock

5. Estimated SY's:
6

Objectives of research:

CRIS-- to identify and chracterize hormones, neurohormones, enzymes, proteins and other chemicals produced by arthropods which affect livestock and determine their role and effects as well as the effects of toxicants, sterilants and other chemicals on insect morphology, physiology and biochemistry.

7. Research priorities in your program:

localize and characterize vertebrate-like peptides within insects under investigation
localize and characterize peptides identified in roach in dipteran pests of livestock
determine target organs of these peptides
determine means of interupting synthesis, release or activity of these peptides

8. Progress of current research in solving problems:

have developed radioautographic and silver localization techniques for detection of specific structures and compounds at the ultrastructural level.
currently attempting to trace specific sites of origin of specific neurosecretion by cobalt backfilling
currently attempting to develop a technique for localization of specific peptides by immunocytochemistry at the EM level.

9. Significant research accomplishments of past 3 years

a. determined the ultrastructure of the major cephalic neurohemal organ(corpus cardiacum) of the stable fly and tsetse fly, and that the intrinsic cells of these organs produced uniqukly formed granules.

b. determined by autoradiography that the insect growth regulator, diflubenzuron, prevented histogenesis of adult epidermis of the stable fly, by inhibition of DNA synthesis.

c. determined that the accessory gland of the male stable fly stores the secretion in villar-lined canaliculi similar to those of parietal cells of mammalian digestive system, and syntheis of this material was not blood meal dependent.

d. in collaboration with scientists of the Texas A&M Rice Laboratory, Beaumont, TX found susceptibility of rice to invasion by Angoumois grain moth was based on morphological differances in absiccion scar of strains of riee.

e. determined that the peritrophic sac of the adult stable fly contains an endogenous bacteria. This was the first finding of a possible bacterial symbiot in the gut of a dipteran that has a free-living larval stage.

f. determined that the insect growth regulator, diflubenzuron prevents formation of melanosomes in melanotic melanoma cells, and cultured murine melanoma cells treated with this compound are unable to induce tumor formation when introduced into mice.

g. using scanning electron microscopy, discovered that bridges occur between red blood cells at the onset of acute kidney dysfunction in sheep and swine, thus being an early diagnostic indicator of acute kidney dysfunction.

10. Impact of research accomplishments on science and the general public
The necessity of aquiring knowledge of the basic morphology, biochemistry and physiology of pest insects is important in developing new methods of approach in the control of these specie Comparative studies of the neuroendocrine systems of insects will indicate similarities and differences that can be exploited in development of methods of interupting this major system of integration of physiological activities in these pests. Also it is imperative to be aware of the areas of similarities and differences between the neuroendcrine systems of vertebrates and insects.

10. Impact (cont.)
The potential of utilization of insect growth regulators in cancer therapy is signifi cant especially if these compounds show low levels of mammalian toxicity as in the case of diflubenzuron.

Aside from sterile-male techniques in use, little knowlege has been aquired of the male reproductive system as an area of insect control. The recent discovery by Dr. Loeb of steroidgenesis in an insect testes shows the need for comparative studies of the endocrine systems of the males of pest species of insects.

The discovery of an endogenous colony of bacteria in the digestive system of the stable fly clearly indicates a new means of approach in the control of this species, especially since this appears to be a species specific microbe.

Determination of the basis of resistance of strains of rice to pest insects of grain is of value in development of new strains of insect resistant rice.

The advantage of discovering a new, rapid indicator of early stages of acute kidney dysfunction, is self-evident.

11. Obstacles to achieving objectives

lack of funds for materials as well as new instruments
need for technical assistance
lack of funds for training in new techniques at other laboratories and for collaboration with other scientists.

12. Future lines of needed research and plan for implementation
a. the use of highly sensitive techniques for localization of both neuropeptides and endocrine proteins in vitro
ei. immunocytochemical and other ultrastructural and histochemical means of detection of and quantitation of these substances in situ in untreated and test animals. This is especially necessary in testing various methods of blocking the activity of the neuroendocrine system of insects as well as in the development of bioassays for these tests.

13. Research Facilities and personnel needs
Laboratory needs to be remodeled to be more effecient for microscopical use (chemical benches need to be replaced by lower benches at which people could use microscopes).

12 year old scanning electron microscope needs to be replaced by a higher resolution instrument, which is easier to maintain and for which parts will be accessable for purchase.

need a part-time assistant to help GS-8 microbiology tichnician who is responsible for specific research projects as well as service research.

14. Extent of cooperation--names of persons and institutions

Dr. Peter Langely, University of Bristol, Bristol England
cooperative reserch on Tsetse fly(Glossina morsitans)
neuroendocrinology

Drs. H. Duve and A. Thorpe University of London, England
cooperative research on immunocytochemistry of several
species of Diptera and vertebrate-like peptides

Dr. Roger Meola, Entomology Dept. Texas A&M Univ.
Cooperative research on neuroendcrine systems of
mosquitoes, stable flies and lepidoptera.

15. Titles of Publications for the last 3 years

Attached

DeLoach, J. R., Meola, S. M., Mayer, R. T. and Thompson, J. M. Inhibition of DNA synthesis by diflubenzuron in pupae of the stable fly, Stomoxys calcitrans (L.). Pesticide Biochem. Physiol. 15: 172-180. 1981.

DeLoach, J. R., Meola, S. M. and Mayer, R. T. Effect of diflubenzuron on thymidine incorporation in Stomoxys calcitrans pupae. Southwest. Entomol. 6(2): 123-125. 1981.

Mayeux, H. S., Jr., Jordon, W. R., Meyer, R. E. and Meola, S. M. Epicuticular wax on goldenweed (Isocoma spp.) leaves: Variation with species and season. Weed Sci. 29: 389-393. 1981.

Meola, S. M. and Meola, R. W. Cephalic Neurohemal Organs of Lepidoptera. Chapt. 12, pp. 393-421. In Gupta, A. P. (ed.) Cephalic Neurohemal Organs of Arthropods, Charles C. Thomas Publ. Co., Springfield, Ill. 642 pp. 1982. (Book Chapter)

Meola, S. M. and Tavares, I. I. Ultrastructure of the haustorium of Trenomyces histophthorus and adjacent host cells. J. Invert. Pathol. 40: 205-215. 1982.

Meola, S. M. Morphology of the region of the ejaculatory duct producing the male accessory gland material in the stable fly, Stomoxys calcitrans L. (Diptera: Muscidae). J. Insect Embryol. Morphol. 11(1): 69-77. 1982.

Meola, S. M., Rowe, L. D., Lovering, S. L. and Steel, E. G. Cytoplasmic Bridges Between Red Blood Cells: An Early Indication of Kidney Dysfunction, pp. 1229-1235. In Scanning Electron Microscopy, Vol III, Scanning Electron Microscopy, Inc., Chicago. 667 pp. 1982. (Book Chapter)

Norman, J. O. and Meola, S. M. Inhibition of melanogenesis in B16-F1 melanoma cells after exposure to diflubenzuron. Antimicrob. Agents & Chemother. 23(2): 313-316. 1983.

Meola, S. M. The structural elements of insect neuroendocrine systems. Southwest. Entomol. Suppl. #5: 9-12. 1983.

Cook, B. J. and Meola, S. M. Heart structure and function of the stable fly, Stomoxys calcitrans. Physiol. Entomol. 8: 139-149.

Cogburn, R. R., Bollich, C. N. and Meola, S. M. Factors that determine relative resistance of rough rice to the Angoumois grain moth and to the lesser grain borers. Environ. Entomol. 12(3): 939-942. 1983.

Bosworth, A. B., Meola, S. M. and Olson, J. K. The chorionic morphology of eggs of Psorophora confinnis complex in the United States. I. Taxonomic considerations. Mosquito Systematics 15(4): 285-309. 1983.

Cook, B. J., Holman, G. W. and Meola, S. M. The oviduct musculature of the cockroach Leucophaea maderae and its response to various neurotransmitters and hormones. Arch. Insect Biochem. & Physiol. 167-178. 1984.

Meola, S. M. Morphology of the neuroendocrine elements of the retrocerebral system of the adult stable fly, Stomoxys calcitrans, pp. 435-437. In A. B. Burkovee and T. J. Kelly (eds.), Proceeding International Conference on Insect Neurochemistry and Neurophysiology, Plenum Publ. 1984.

Norman, J. O., Thompson, J. M., Spates, G. E. and Meola, S. M. Identification and ultrastructure of bacteria found in the midgut of the stable fly, Stomoxys calcitrans (L.) Southwest. Entomol. 9(2): 151-157. 1984.

Costello, D., Meola, S. M., and Odom, T. W. Observations of the effects of eggshell pimpling on shell ultrastructure. (Accepted by Poultry Sci., April 1984)

1. Scientist's name, address, and telephone number:

Renée M. Wagner, Research Chemist
USDA, ARS, SPA, VTERL
P.O. Drawer GE
College Station, TX 77841
COMM: 409-260-9315 FTS: 527-1315

2. Location:

Veterinary Toxicology and Entomology Research Laboratory
College Station, Texas

3. Number and title of CRIS work unit:

6202-20482-001 - Physiological and toxicological studies of those arthropods that affect livestock

4. Approach Element and Problem Definitions:

3-5-03-1a PRODUCTIVITY/QUALITY--ANIMALS
CONTROL OF INSECTS, TICKS, & MITES
REDUCE LOSSES-ANIMALS & MAN

5. Estimated SY's:

6 - One not working on neuropeptides

6. Objectives of research:

Identify and characterize hormones, neurohormones, enzymes, proteins, and other chemicals produced by arthropods that affect livestock and determine their role and effects, as well as the effects of toxicants, sterilants, and other chemicals on insect morphology, physiology and biochemistry.

Special equipment and technical capabilities:

Equipment
1. Gas-phase protein sequencer
2. FAB mass spectrometer (part-time accessibility, located outside of unit)

Capabilities/expertise
1. Manual peptide synthesis and synthesis of peptide-drug conjugates
2. Peptide sequencing: manual Edman degradation, enzymatic/HPLC mapping, automated Edman degradation, peptide structure determination by FAB mass spectrometry, microsequencing techniques.

7. Research priorities in your program:

(1) Metabolism of livestock insects and neuroendocrine modulation of metabolism. These metabolic processes include amino acid metabolism, carbohydrate metabolism and excretion.
(2) Endocrine control of development.

8. Progress of current research in solving problems:

(1) Developed enzymatic sequencing method for insect neuropeptides.
(2) Identified molecular weight and partial structure of several myogenic insect peptides.

9. Significant research accomplishments in the past 3 years:

Limited to 2 years: 1 year post-doctoral research associate plus 1 year career conditional.

a. Developed enzymatic/mass spectrometric technique for complete peptide sequence determinations.
b. Developed mass spectrometric assay for selection of peptides containing methionine and tryptophan.
c. Studied consequences of chemical modifications of proteins on sequence analysis.

10. Impact of research accomplishments on science and the general public:

a. Assay for Met and Trp: facilitate selection of sequence of protein for synthesis of a DNA probe.
b. Consequences of chemical modifications: Alert scientists to ways in which they may be nullifying the results of their research (e.g., in preparation of "homogenous" class of antibodies).

11. Obstacles to achieving objectives:

Research personnel, support personnel, limited equipment needs, renewal of increased funding for support and upkeep of new equipment.

12. Future lines of needed research and plan for implementation:

Future research:

a. Isolation of polypeptides which are precursors to active neuropeptides now being purified and sequenced. Study of degradation/processing of precursors to active forms.
b. Isolation of mRNA or DNA coding for precursor peptides. Study of regulation of DNA processing to mRNA and control of translation of mRNA to peptide products.

Implementation:

Acquisition of post-doctoral research associate familiar with immunological or molecular biological techniques to initiate these experiments and to set up protocols for the later work.

13. Research facilities and personnel needs:

PERSONNEL:

a. Support personnel for peptide synthesis and sequencing.
b. Associate research personnel to develop antibodies to insect peptides and to isolate mRNA responsible for coding of peptide sequence.

RESOURCES:

a. Funding to support equipment already present (i.e., chemicals and materials).
b. Peptide synthesizer or means of obtaining a large number of custom-synthesized peptides.

14. Extent of cooperation--names of persons and institutions:

Cooperative Efforts:

V. Sylvia - Texas A&M University (TAMU)
J. Mullet - TAMU
B. Fraser - Food and Drug Administration
H. Jaffe, A. Raina - USDA
T. Hayes - TAMU (advisory capacity only)

15. Titles of publications for the last 3 years:

SEE ATTACHED